EUROPEAN HEMATOLOGY ASSOCIATION

haematologica

Journal of the European Hematology Association
Published by the Ferrata Storti Foundation

46th Congress of the Italian Society of Hematology
Rome, Italy, October 15-18, 2017

ABSTRACT BOOK

ISSN 0390-6078

Volume 102
OCTOBER
2017|s3

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Haematologica (print edition, pISSN 0390-6078, eISSN 1592-8721) publishes peer-reviewed papers on all areas of experimental and clinical hematology. The journal is owned by a non-profit organization, the Ferrata Storti Foundation, and serves the scientific community following the recommendations of the World Association of Medical Editors (www.wame.org) and the International Committee of Medical Journal Editors (www.icmje.org).

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Rates of the International edition for the year 2017 are as following:

	Institutional	*Personal*
Print edition	*Euro 500*	*Euro 150*

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Direttore responsabile: Prof. Edoardo Ascari; Autorizzazione del Tribunale di Pavia n. 63 del 5 marzo 1955.
Printing: Tipografia PI-ME, via Vigentina 136, Pavia, Italy. Printed in Agosto 2017.

EBAH
European Board for Accreditation in Hematology

From EHA-CME to EBAH

Fueled by the rapid progress in medical science and spectacular advances in technology and bioengineering made over the last decades, the specialization of hematology is a strong and rapidly evolving scientific and medical discipline.

Hence it is essential to ensure that hematologists attend high-quality educational programs. In response to this need, the European Council for Accreditation in Hematology (ECAH) was established in 2003, funded by the European Commission. Following the completion of the ECAH project, the EHA-CME Unit was then established in 2005 and has been accrediting high quality educational events ever since.

In over 10 years of continuous efforts to promote high standards and high quality in CME practices in hematology, we have constantly worked on further developing ourselves. And now the time has come to update our name so that it reflects more clearly the accreditation role we take on in the hematological community.

We are proud to announce the change from EHA-CME Unit to the **European Board for Accreditation in Hematology (EBAH)**. We believe this name reflects our activities better and it is also in line with other specialty accreditation boards in Europe.

With strict guidelines and thorough review that considers the specificities in the hematology field, we have gained high level expertise and efficient procedures. EBAH supports hematologists in making the choice of which educational activities to participate in.

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EBAH - Stay on course with your professional development!

Note: system log-in remains the same

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Shaping the future of mesenchymal stromal cells therapy

Dates: November 23-25, 2017
Location: TBC

Organized by:
EHA Scientific Working Group on Mesenchymal stromal cells

Chair:
WE Fibbe

Co-chairs:
F Dazzi, K Le Blanc

Topics:

- Basic developmental biology of MSC and their role in immune regulation
- Mechanisms of MSC immune regulation
- Potency assay design
- Technological development
- Clinical trial results with MSC
- Clinical issues
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The origin of a name that reflects Europe's cultural roots.

Ancient Greek

αἷμα [haima] = blood
αἵματος [haimatos] = of blood
λόγος [logos]= reasoning

Scientific Latin

haematologicus (adjective) = related to blood

Scientific Latin

haematologica (adjective, plural and neuter, used as a noun) = hematological subjects

Modern English

The oldest hematology journal, publishing the newest research results.
2016 JCR impact factor = 7.702

Haematologica, as the journal of the European Hematology Association (EHA), aims not only to serve the scientific community, but also to promote European cultural identify.

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ABSTRACT BOOK

46° Congress of the Italian Society of Hematology
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BEST ABSTRACTS

B001

INTERIM ANALYSIS OF CARFILZOMIB-LENALIDOMIDE-DEXAMETHASONE *VS* CARFILZOMIB-CYCLOPHOSPHAMIDE-DEXAMETHASONE IN THE FORTE TRIAL

F. Gay[1], A. Spadano[2], M. Cavo[2], T. Caravita[2], L. Canepa[2], N. Giuliani[2], S. Spada[1], F. Patriarca[2], F. Morabito[2], P. Tacchetti[2], F. Narni[2], G. De Sabbata[2], F. De Santis[1], A. Pascarella[2], S. Palmieri[2], A.M. Liberati[2], F. Pisani[2], M. Genuardi[1], P. Tosi[2], O. Annibali[2], M. Ruggeri[2], P. Curci[2], L. De Rosa[2], A. Palumbo[3], P. Musto[2], M. Boccadoro[1]

[1]Myeloma Unit, Division of Hematology, University of Torino, Torino, Italy, [2]Italian Multiple Myeloma Network, GIMEMA, Italy, [3]Myeloma Unit, Division of Hematology, University of Torino, Italy – Currently Takeda PharmaceuticalsCo, Torino, Zurich

Background: Carfilzomib-Lenalidomide-Dexamethasone (KRd) and Carfilzomib-Cyclophosphamide-Dexamethasone (KCd) showed to be safe and effective in newly diagnosed multiple myeloma (NDMM) patients (Jakubowiak Blood 2012, Bringhen Blood 2014). The FORTE study assessed KCd *vs* KRd in patients eligible for transplantation. Here we describe results of the first planned safety interim analysis on induction and mobilization. Preliminary efficacy data are also reported. *Methods:* NDMM patients <65 years were included. Patients were randomized (1:1:1; stratification ISS and age) to: 4 28-day KCd cycles (carfilzomib:20/36 mg/m^2 IV d 1, 2, 8, 9, 15, 16; cyclophosphamide 300 mg/m^2 d 1, 8, 15; dexamethasone: 20 mg d 1, 2, 8, 9, 15, 16) followed by high-dose melphalan and autologous stem cell transplantation (MEL200-ASCT) and consolidation with 4 KCd cycles; or 4 28-day KRd cycles (carfilzomib and dexamethasone as above; lenalidomide:25 mg d 1-21) followed by MEL200-ASCT and 4 KRd cycles; or 12 KRd cycles. All patients received Cyclophosphamide 2 g/m^2, followed by peripheral blood stem cell collection after the 4th induction cycle. For this interim analysis, data of the two KRd groups were pooled together, as patients had in fact received the same treatment until mobilization. Data cut-off was October 30, 2016.

Table 1.

Grade 3-4 AEs/SAEs	KCd	KRd
Hematologic	13%	9%
Cardiac	1%	2%
Hypertension	0%	2%
Thromboembolism	0%	1%
Gastrointestinal	0%	3%
AST/ALT/GGT increase	1%*	7%*
Dermatological	0%*	7%*
Infections	6%	9%
Acute Kidney Injury	0%	2%

*p value <0.05

Results: 281 patients were evaluated (94 received KCd and 187 received KRd). Hematologic adverse events (AEs) (mainly neutropenia) and infections (mainly pneumonia/fever) were the most common grade 3-4 AEs and serious AEs (SAEs) in both arms; increased AST/ALT/GGT (mainly reversible) and dermatological (rash) AEs were more frequent with KRd; cardiac AEs were 2% with KRd (including atrial fibrillation [1%] and ischemic heart disease[1%]) *vs* 1% with KCd (atrial fibrillation). 1 patient died in the KCd group (infection not treatment-related) *vs* 3 patients in the KRd group (2 cardiac arrest [1 not treatment-related], 1 infection not treatment-related). In the KCd *vs* KRd arms, 99% *vs* 95% (P=0.44) of patients mobilized stem cells (median number of PBSC collected: 9 *vs* 6x10^{6}CD34/Kg with KCd *vs* KRd). Plerixafor was required in 10% *vs* 24% (P=0.01), respectively. At least a very good partial response (VGPR) rate was 61% with KCd *vs* 74% with KRd (P=0.05) (Table 1). *Conclusions:* Safety of both combinations was acceptable; plerixafor was needed in more patients in the KRd arm, VGPR rate was higher with KRd. Updated data will be presented at the meeting.

B002

SEIFEM 2015-B: INCIDENCE AND MORTALITY FOR CANDIDEMIA IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

G. Dragonetti[1], C. Cattaneo[2], F. Marchesi[3], F. Aversa[4], A. Busca[5], A. Candoni[6], C. Castagnola[7], S. Cesaro[8], M. Criscuolo[1], M.I. Del Principe[9], N. Decembrino[10], M. Delia[11], R. Fanci[12], A. Ferrari[13], N.S. Fracchiolla[14], F. Lessi[15], V. Mancini[16], B. Martino[17], L. Melillo[18], G. Nadali[19], A. Nosari[16], K. Perruccio[20], M. Picardi[21], L. Prezioso[22], M. Stanzani[23], M. Tumbarello[1], B. Veggia[24], L. Pagano[1] on the behalf of the SEIFEM group

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Aims: To evaluate changes in the epidemiology of Candidemia in Hematological Malignancies (HMs) in the era of new antifungals. *Methods:* In this retrospective cohort study, was analyzed the incidence and mortality of Candidemia among HMs and patients underwent to autologous (auto-HSCT) or allogeneic HSCT (allo-HSCT), in the era of echinocandins therapy and azole prophylaxis. The registry collected data, between 2011 and 2015, on patients with acute myeloid or lymphoid leukemia (AML and ALL, respectively), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM), treated with conventional chemotherapy and also patients who underwent to autologous auto-

HSCT or allo-HSCT, admitted to 23 hematologic divisions in tertiary care centers or university hospitals in Italy, who developed a positive blood colture for Candida spp. during the study period. *Results:* Overall 16,529 HMs were admitted in the participating centers for conventional chemotherapy (4581 AMLs, 954 ALLs, 8542 NHLs, 2542 MMs). 135 patients developed a candidemia. The overall incidence was 0.8%. In the different subsets of patients the incidence ranged from 0.3% among MMs to 1,6% in ALLs. Among 135 patients with candidemia 62% of infections occurred in patients with acute leukemia (51% in AML, 11% in ALL). The attributable mortality (AM) was 0.2% (30/16,259) ranging from 0.2% in MM to 0.3% in AML. The case fatality rate in patients with candidemia was 22% (30/135), ranging from 0% in ALL to 75% in MM. In the same period of observation, 6,928 patients received a HSCT (4338 auto-HSCTs and 2,590 allo-HSCTs). Overall 59 patients developed a candidemia with an incidence of 0.8%, 21 among auto-HSCTs (0.5%) and 38 among allo-HSCTs (1.5%). The attributable mortality for candidemia was 0.002% (11/6,928), respectively 0.04% in auto-HSCT (2/4338) and 0.3% (9/2590) in allo-HSCT. The overall case fatality rate in patients with candidemia was 19% (11/59); 9.5% (2/21) in auto-HSCT and 25% (9/38) in allo-HSCT respectively. *Conclusions:* At present the overall incidence and mortality of Candidemia, in patients treated with conventional chemotherapy, was significantly reduced, and it was markedly influenced above all by a relevant reduction of Candidemia in AMLs, probably because of more effective prophylactic and/or therapeutic antifungal approaches (posaconazole). In transplanted patients no differences were observed for overall incidence, while a significant lower mortality rate was observed only in auto-HSCTs.

B003

RISK-ADAPTED, MRD-DIRECTED THERAPY FOR YOUNG ADULTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA: RESULTS OF THE AML1310 TRIAL OF THE GIMEMA GROUP

A. Venditti*, A. Piciocchi, A. Candoni, L. Melillo, V. Calafiore, R. Cairoli, P. De Fabritiis, G. Storti, P. Salutari, F. Lanza, G. Martinelli, M. Luppi, P. Mazza, B. Falini, A. Cuneo, G. Specchia, F. Fabbiano, A. Tafuri, B. Ronci, A. Tieghi, N.S. Fracchiolla, D. Capelli, R. Foà, F. Ronco, E. La Sala, P. Fazi, L. Maurillo, F. Buccisano, M.I. Del Principe, F. Lo Coco, W. Arcese, S. Amadori

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From January 2012 to May 2015, 515 pts with *de novo* AML, 18 to 60 years old, seen at 55 GIMEMA institutions were enrolled in the AML1310 trial. All pts in CR/CRi after 1-2 induction cycles, received 1 consolidation course. In those with ELN low- or intermediate-risk profile, PBSC collection was attempted by initiating, on day 20 from the start of consolidation therapy, G-CSF until completion of stem cell collection. After consolidation, low-risk pts (NPM1 positive FLT3-ITD negative or CBF positive without c-Kit mutations) received AuSCT; high-risk pts (adverse karyotype or FLT3-ITD positive) received ASCT; intermediate-risk pts (intermediate karyotype or FLT3-TKD positive or c-kit mutated CBF positive) received AuSCT or ASCT depending on the levels of MRD, measured by flow cytometry after consolidation. Five-hundred of 515 pts were available for the analysis. Median age was 49 (18-61) years and 52% were males. Of 429 evaluable pts, ELN risk-category was: low 11%, intermediate 73% and adverse 16%. RUNX1/RUNX1T1 was detected in 5% of 499 evaluable cases, CBF-beta/MYH11 in 7% of 496, FLT3-ITD in 25% of 497 and NPM1 in 37% of 499. In 494 evaluable pts, CR rate was 73% (361), 18% had refractory AML and 9% died early during induction. Three hundred-41 pts completed the consolidation phase and were risk allocated: 114 (33%) to the low-risk category (=AuSCT), 122 (36%) to the high-risk (=ASCT) and 78 (23%) to the intermediate category (=AuSCT or ASCT). In 27 pts (8%) belonging to the intermediate-risk category, a leukemia associated phenotype was not found and they were to receive AuSCT. Overall, 109 (33%) and 123 (36%) of 341 pts received AuSCT and ASCT, respectively. Median follow-up was 27.9 months. At 24 months, OS and DFS of the whole series was 55.9% and 54.9%, respectively; CIR was 32.9%. OS and DFS in the low-risk category was 74.8% and 63.8%, respectively; in the high-risk category 42.5% and 44.8%, respectively; in the intermediate-risk category MRD negative 78.6% and 61.4%, respectively; in the intermediate-risk category MRD positive 69.8% and 66.6%, respectively. In conclusion, a program of risk-adapted, MRD-driven therapy is feasible in a multicenter, cooperative setting. In the intermediate-risk category, ASCT can be avoided if MRD is not detectable; if MRD is positive, ASCT can prolong OS and DFS to equalize those of the low-risk category. ASCT was delivered to 2/3 of pts in the high-risk category, using all the available sources of stem cells (Figure 1).

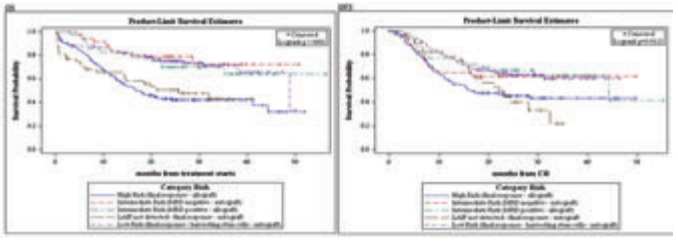

Figure 1.

B004

CLINICAL IMPACT OF TP53 AND KMT2D MUTATIONS IN MCL RECEIVING HIGH-DOSE THERAPY AND AUTOLOGOUS TRANSPLANTATION: UPDATED RESULTS FROM THE FONDAZIONE ITALIANA LINFOMI MCL0208 PHASE III TRIAL

S. Ferrero[1], D. Rossi[2], A. Bruscaggin[3], A. Evangelista[4], A. Di Rocco[5], V. Spina[3], V. Stefoni[6], P. Ghione[1], D. Barbero[1], L. Monitillo[1], M. Gomes da Silva[7], A. Santoro[8], A. Molinari[9], A. Ferreri[10], A. Piccin[11], S. Cortelazzo[12], M. Ladetto[13], G. Gaidano[14]

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In mantle cell lymphoma (MCL), only TP53 disruption has been so far associated with outcome. We present the clinical update of the deep sequencing gene panel analysis in the FIL-MCL0208 phase III trial (NCT02354313, high-dose immunochemotherapy followed by autologous transplantation for untreated, advanced stage <65 years MCL) based on the data from the second interim analysis. A targeted resequencing gene panel, including coding exons and splice sites of the ATM, BIRC3, CCND1, KMT2D, TP53, TRAF2, WHSC1, and NOTCH1 genes was analyzed in tumor DNA from bone marrow CD19+ purified cells and, to filter out polymorphisms, in the paired normal genomic DNA using a TruSeq Custom Amplicon target enrichment system followed by deep next generation sequencing (Illumina, median depth of coverage 2356x). Variants represented in >10% of the alleles were called with VarScan2 with the somatic function when the paired germline DNA was available. For patients lacking germline DNA, a bioinformatics pipeline with stringent filters was applied to protect against misclassifications. Clinical data were updated as of January, 2017. 174/300

patients were evaluable for mutations. Median follow-up was 36 months, and 3-years PFS and OS were 67% and 86%, respectively. Patients not included, due to unavailable tumor DNA (n=126) showed the same features and outcome. Mutations of TP53 (8%) and KMT2D (11%) associated with an increase in the hazard of progression both in univariate analysis as well as after adjusting for MIPI, Ki67 and blastoid variant: HR 3.87 (95% CI 1.64 to 9.13), p<0.002 and HR 3.66 (95% CI 1.77 to 7.56), p<0.001, respectively. This translated into an increase of the hazard of death in both TP53 and KMT2D mutated patients both in univariate analysis as well as adjusting for MIPI, Ki67 and blastoid variant HR 4.26 (95% CI 1.34 to 13.57), p=0.014 and HR 3.09 (95% CI 1.07 to 8.86), p=0.036, respectively. A survival model was proposed based on TP53 and KMT2D mutations: 3-years PFS and OS were 26% and 64% for patients carrying either mutations or both *vs* 75% and 92% for patients without any of these (Figure1). The updated clinical results show that: i) both TP53 and KMT2D mutations independently associate with shorter PFS and OS in MCL receiving high-dose therapy; ii) KMT2D seem to be as detrimental as TP53 mutations, at least in terms of PFS; iii) given the negative prognostic impact, they might be used to select high-risk patients for novel therapeutic approaches.

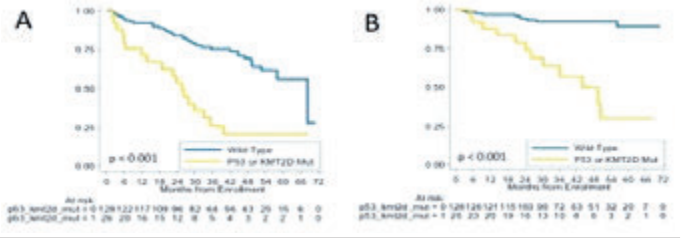

Figure 1. PFS (A) and OS (B) by TP53 and/or KMT2D mutational status.

B005

GENE THERAPY FOR BETA THALASSEMIA: INITIAL RESULTS FROM THE PHASE I/II TIGET-BTHAL TRIAL OF AUTOLOGOUS HEMATOPOIETIC STEM CELLS GENETICALLY MODIFIED WITH GLOBE LENTIVIRAL VECTOR

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Gene therapy for transfusion dependent beta-thalassemia, as an alternative cure to allogeneic HSCT, is based on the autologous transplantation of hematopoietic stem cells (HSCs) engineered by lentiviral vectors expressing a transcriptionally regulated human beta-globin gene. Our contribution to this field was devoted to the clinical development of a gene therapy protocol based on high-titer vector GLOBE, use of lenograstim and plerixafor as source of HSCs and a conditioning regimen based on myeloablative treosulfan and thiotepa favoring efficient engraftment of corrected cells with reduced toxicity (TIGET-BTHAL; EudraCT number 2014 004860 39). On the basis of extensive efficacy and safety preclinical studies the clinical trial TIGET-BTHAL was approved and started in 2015 at Scientific Institute San Raffaele, Milan, Italy. The clinical study foresees treatment of 10 patients: 3 adults followed by 7 minors, with a staggered enrolment strategy based on evaluation of safety and preliminary efficacy in adult patients by an independent data safety monitoring board before inclusion of pediatric subjects. The chosen route of administration of gene modified HSCs is intraosseous in the posterior-superior iliac crests, bilaterally, with the aim of enhancing engraftment and minimizing first-pass intravenous filter. As of April 2017, seven patients (3 adults and 4 pediatric patients) with different genotypes (0/ 0, +/+and 0/ +) have been treated with GLOBE-transduced CD34+ cells at a dose of 16x106-19.5x106 cells/kg and a vector copy number (VCN)/cell ranging from 0.7 to 1.5. The procedure was well tolerated by all patients, with no product-related adverse events. Multilineage engraftment of gene-marked cells was observed in all tested peripheral blood and bone marrow samples. Polyclonal vector integrations profiles have been detected in the first 3 patients tested. So far, the clinical outcome indicates reduction in transfusion requirement in adult patients and greater clinical benefit in younger patients. Follow up analysis are ongoing and updated clinical outcome will be presented.

B006

CYTOGENETIC ABNORMALITIES IN POST-POLYCYTHEMIA VERA AND POST-ESSENTIAL THROMBOCYTHEMIA MYELOFIBROSIS: CORRELATIONS WITH GENOTYPE AND PHENOTYPE IN THE MYSEC STUDY

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Background: Information about molecular and phenotypic correlates of cytogenetic abnormalities in secondary myelofibrosis (SMF) is scant. The MYSEC project (Myelofibrosis Secondary to PV and ET Collaboration) on 781 SMF patients disclosed phenotype-genotype associations in SMF (Leukemia, 2017). *Aims:* To assess distribution and molecular, phenotypic and prognostic correlations of cytogenetic abnormalities in SMF. *Methods:* Marrow cytogenetic analysis was made at time of SMF diagnosis. Karyotype was defined abnormal if a structural or numeric chromosomic alteration was present in at least two metaphases. Three or more aberrations defined a complex karyotype (CK); two or more autosomal monosomies or single autosomal monosomy associated with at least one structural abnormality defined monosomal karyotype (MK). Continuous values were compared via non-parametric Mann-Whitney U tests; categorical feature counts were compared with Fisher's exact tests. Time-to-event analysis used

Kaplan-Meier estimators and Cox models for regression. *Results:* Within the MYSEC cohort, 376 patients had cytogenetic data: 128 (34.1%) showed cytogenetic abnormalities, of whom known 72 (60%) sole, 22 (18.3%) double and 26 (21.7%) CK. MK was found in 11 CK cases (8.6%). The most frequent sole abnormalities were 20q- (25%), 13q- (20.8%), +8 (8.3%) and +9 (5.6%). Patients with post-PV MF had significantly higher frequency of abnormal karyotype than those with post-ET MF (P=.012). Chromosomal abnormalities did not cluster differently among genotypes (JAK2, CALR, MPL and triple negativity). Abnormal karyotype was significantly associated with lower platelets (P=.004), larger spleen size (P=.016), higher circulating blasts (P < .001) and constitutional symptoms (P=.014) at time of SMF diagnosis. Compared to patients with sole aberrations, those with MK have lower platelets (P=.04). Overall survival (OS) was significantly inferior in patients with abnormal karyotype (P=.012), even adjusting for SMF diagnosis type (P=.02). Patients with MK have inferior OS respect to those with sole abnormality (P < .0001) (Figure 1). *Summary:* Abnormal karyotype was found in 34.1% of SMF patients and is over-represented in post-PV MF. No different distribution was detected among genotypes. Abnormal karyotype was associated with lower platelet count, larger splenomegaly, higher circulating blasts, constitutional symptoms and inferior survival. Among subtypes, MK remained the most powerful predictor of outcome.

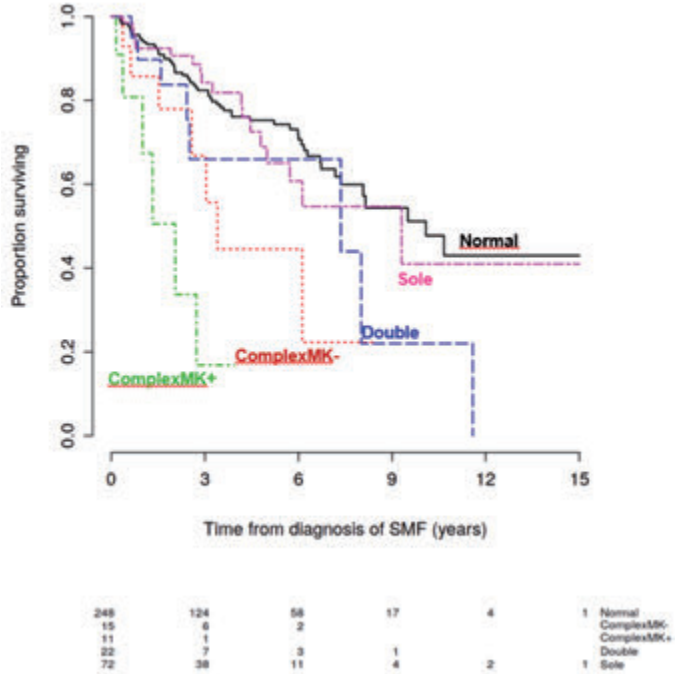

Figure 1. Impact of karyotype on overall survival in patients with post-polycythemia vera and post-essential thrombocythemia.

B007

PROGNOSTIC IMPLICATIONS OF SOMATIC MUTATIONS BY NEXT GENERATION SEQUENCING: AN ANALYSIS FROM THE MMRF COMMPASS STUDY IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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The characterization of multiple myeloma (MM) genomic profiles through next generation sequencing can be helpful to assess patients' baseline risk. However, the relevance of this approach in MM field is not defined yet. In the CoMMpass trial (NCT01454297) clinical and molecular data from 1154 newly-diagnosed MM (NDMM) patients were collected. The analysis of somatic mutations in NDMM cells could reveal disease characteristics not detectable with traditional approaches. Whole exome libraries from CD138+ purified bone marrow MM cells and peripheral blood normal cells were created. Nonsynonymous somatic single nucleotide variants (SNV) in MM cells were identified. The impact on progression free survival (PFS) of recurrently mutated genes was evaluated in a multivariable Cox model. A backward selection based on the Akaike Information Criterion (AIC) was used to select the genes included in the final Cox model used to create a scoring system. 517 NDMM patients with available data at the interim analysis 8 cohort (August 2015) were analyzed. Median age was 64 years, all patients received novel agents as first line treatment, 236 (45.6%) of them received high dose therapy followed by stem cell transplantation. KRAS and NRAS were the genes most frequently affected by a nonsynonymous SNV (25 and 19.5% of cases respectively). As described above, we created a scoring system based on the mutational status of 9 genes (Table 1).

Table 1. Mutational score obtained by adding score for each parameter (total points).

Gene	Mutated Yes/No	Score assigned
PRRC2C	Yes	3
USH2A	Yes	2
RBP3	Yes	2
PKHD1	Yes	2
HRNR	Yes	2
FAT4	No	2
KRAS	Yes	1
FAT3	Yes	1
NRAS	No	1

Additive total score	
Group I	0-2
Group II	3
Group III	4-5
Group IV	>5

4 prognostic groups with different additive scores were identified: group I (score 0-2, 17% of patients); group II (score=3, 51%), and group III (score 4-5, 26%) and group IV (score >5; 6%). After a median follow-up of 1 year, the 18-month PFS was 93% for group I, 85% for group II, 73% for group III and 40% for group IV (Figure 1).

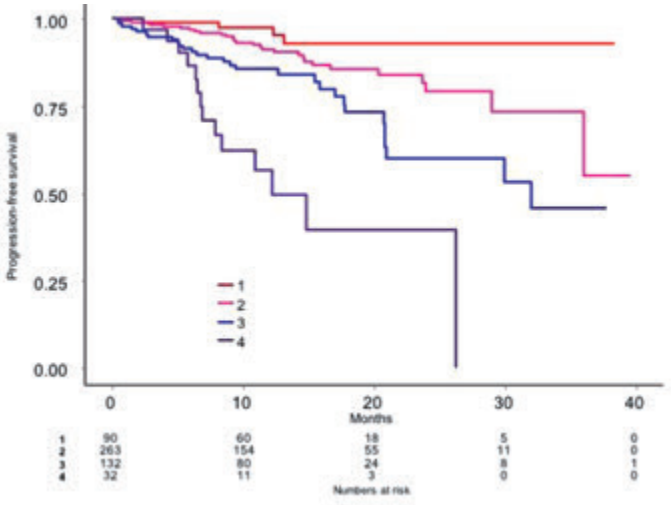

Figure 1. PFS stratified according to mutational score subgroups. Group I (score 0-2, red), group II (score=3, pink), group III (score 4-5, blue), group IV (score >5, purple).

The hazard ratio was 2.31 (p=0.118) for group II *versus* group I, 4.45 (p=0.006) for group III *versus* I and 17.38 (p<0.001) for group IV *vs* I. The prognostic value of the score was consistent across different subgroups

including ASCT/no ASCT, standard/high cytogenetic risk, ISS I, II, or III. Of note, 23% of patients in group I had an ISS III and 34% of patients in group IV had an ISS I. In conclusion, the use of a prognostic tool based on the mutational status of 9 recurrently mutated genes could improve risk assessment of NDMM patients. Longer follow-up and validation in independent cohorts of patients are needed to confirm our findings. Updated results with a longer follow-up will be presented at the meeting.

B008

RETROSPECTIVE PLASMIC SCORE APPLICATION AND VALIDATION IN PATIENTS WITH DIAGNOSIS OF THROMBOTIC THROMBOCYTOPENIC PURPURA

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Acquired idiopathic thrombotic thrombocytopenic purpura (TTP) is a life-threatening microangiopathic disorder, distinguished from the other syndromes characterized by thrombotic microangiopathy, by severe deficiency in the ADAMTS-13 enzyme. Recently PLASMIC SCORE, a clinical prediction tool, has been developed and published to stratify patients with TTP according to their risk of having severe ADAMTS-13 deficiency (Bendapudi PK et al, Lancet Hematology 2017). Seven predictors of severe ADAMTS13 deficiency (<10%) in the PLASMIC SCORE were: platelet count lower than 29 109 per L, creatinine less than 1·8 mg/dL, international normalised ratio (INR) less than 1·3, mean corpuscular volume (MCV) less than 86·5 fL, a combined haemolysis variable judged positive; no history of solid-organ or stem-cell transplant and no active cancer. Patients with score of 6 or 7 had more frequently severe ADAMTS13 deficiency. The aim of our study was to apply and validate retrospectively, PLASMIC SCORE, in a TTP population series treated in our Hematological Department between January 2005 and April 2017. Forty-seven patients [36 female and 11 male (ratio F:M 3:1); median age 42.5 age (range 17-80)] with TTP diagnosis were registered and treated in this period. Thirty-six were naïve patients, at their first episode of TTP, while 12 patients had an history of multiple relapses [median 2 (range 2-4)] in the previous 10 years. Median platelet and Hb values at TTP diagnosis were 13x109/l (range 5-49) and 7,9 g/dl (4,2-10.1) respectively. ADAMTS-13 testing at TTP diagnosis was available in all patients and resulted <10% in all but 1 case, who had ADAMTS-13 activity of 20%. Applying PLASMIC SCORE' variables at our series, 8 patients (17%) resulted with a score of 6 and 39 patients (83%) with a score of 7. The only patient with ADAMTS-13 activity more than 10% had a PLASMIC SCORE of 7. Interestingly no score values lower than 6 were registered in our series. No differences in survival and treatment response was observed between score of 6 or 7. The high score values (6-7) of our TTP population, characterized by severe ADAMTS-13 deficiency in 98% of cases, confirms the predictive power of PLASMIC score to assess reliably the probability of severe ADAMTS13 deficiency in adult patients with thrombotic microangiopathy. Plasmic score, easily applicable, could be useful to differentiate TTP from the other thrombotic microangiopathies, when the results of ADAMTS13 activity testing are not readily available.

B009

CLINICAL CHARACTERISTICS OF PATIENTS WITH NEGATIVE INTERIM-PET AND POSITIVE FINAL PET: DATA FROM THE PROSPECTIVE PET-ORIENTED HD0801 STUDY BY FONDAZIONE ITALIANA LINFOMI

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Introduction: In many prospective studies interim-PET (i-PET) has shown an high prognostic impact in the definition of ABVD efficacy in advanced Hodgkin Lymphoma (HL). Patients (pts) with positive i-PET had a worst prognosis in comparison with those with negative i-PET. In these studies was emerged that there are pts with i-PET negative but with a positive results at the end of therapy (PET6 positive). This group represent the failure of functional imaging in differentiating chemosensitive *versus* not chemosensitive pts. The aim of this multicenter prospective PET-oriented study supported by FIL (HD0801) was to identify i-PET-negative/PET6-positive pts and to define their clinical characteristics, in order to identify earlier pts with worst prognosis despite of negative i-PET. *Methods:* in HD0801 study, pts with advanced Hodgkin Lymphoma performed PET at staging after 2 cycles of ABVD (i-PET) and at the end of therapy (PET6) to define response to therapy. *Results:* Between September 2008 and April 2013, 520 pts were enrolled and 512 performed i-PET. Four hundred and nine were i-PET negative and continued ABVD, 16 pts interrupted therapy before end of therapy, 3 of these due to disease progression; the remaining 393 pts performed, after 6 ABVD, final PET to define response to therapy (PET6). In 355 PET6 was negative and in 38 pts it was positive confirming a progression of the disease. In summary pts with progressive disease after negative i-PET were 41: 38 (PET6 positive) and 3 progressed between i-PET and PET6. None of the analyzed clinical characteristics were significantly different between 355 PET6 negative and 41 PET6 positive a part for LDH value at diagnosis either in univariate or in multivariate analysis: Odds Ratio (OR) 2.33 (1.15 to 4.7). The only factor predicting OS in the group of pts with i-PET negative was the positive results of PET6 with an OR 83.74 (12.75 to 557.64). The OS from PET6 at 24 and 36 months was 99% and 98% for PET6 negative pts and 91% and 78% for PET6 positive pts. *Conclusions:* pts with negative i-PET but with a positive PET at the end of therapy had a very bad prognosis even in comparison with i-PET positive pts salvaged with intensification of therapy. From our study only LDH value at diagnosis was associated with a significant probability to have a positive PET6. Probably biological and pathological markers could be associated with i-PET to increase the predictive power but in particular to reduce the false negative i-PET.

B010

MICROBIOME-DERIVED MARKERS PREDICT THE CLINICAL OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

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Recent advances in supportive care have significantly reduced TRM; nevertheless infections and GvHD represent major complications in allo-HSCT. Recent studies indicate that patients undergo dramatic alterations of intestinal microbiota during allo-HSCT, potentially affecting the outcome. Between October 2014 and March 2016, we conducted a prospective observational study to examine the intestinal microbiota by NGS techniques in 100 consecutive adult patients, who received allo-HSCT for high-risk hematological malignancies. Fecal specimens were collected before conditioning (T0), during aplasia (T10) and after engraftment (T30). The transplant procedures markedly impacted the enteric microbiome, with a dramatic decrease of the intestinal microbial diversity (alpha diversity), especially between T0 and T10 ($p<0{\cdot}0001$). The presence of ≥ 5% Proteobacteria, and in particular of Enterobacteriaceae, at T0 was the most sensitive and specific risk-stratification marker for clinical outcomes. Patients who developed sepsis by GN-MDR bacteria

show an increase of Enterobacteriaceae (cut-off 5%; p= 0·001, RR 5·6). This increase of Enterobacteriaceae was significant also when considering severe sepsis and septic shock (RR 2·125; p= 0·0425). These microbiome changes was significantly associated to OS (RR 2·541; p= 0·0001), confirmed by the multivariate analysis. A low (<=10%) amount of Lachnospiraceae at T0 is associated to an increased risk of GN-MDR sepsis (p= 0·0261), whereas a <=10% amount of Ruminococcaceae to increased risk of severe sepsis and septic shock (p= 0·0259). Both markers were associated to increased risk of death (p= 0·0001 and p= 0·0404). More in details, <=10% Lachnospiraceae was associated to an increased risk of death for infectious and non-infectious causes (p= 0·0002). Interestingly, significant microbioma changes were observed 10 days after transplant, in patients who developed acute GvHD, with a predominant role played by gram-positive bacteria belonging to Firmicutes. The presence of Lachnospiraceae was associated to a decreased risk of developing acute GvHD (p=0·04 and RR= 4·35), whereas dominance of Enterococcaceae (p<0·01 and RR=3·23) and Staphylococcaceae (p<0·01 and RR=3·5) was associated to its increased incidence. Longitudinal study of microbiome allows early identification of patients at risk for major transplant-related complications, offering a new tool for individualized therapeutical strategies to improve the outcome of allo-HSCT.

B011

FRONT-LINE THERAPY WITH FLUDARABINE, CYCLOPHOSPHAMIDE, OFATUMUMAB (FC-O2) IN YOUNG (≤65 YRS) AND FIT CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS. RESULTS OF THE PROSPECTIVE PHASE 2 GIMEMA STUDY LLC0911

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The fludarabine, cyclophosphamide, rituximab (FC-R) regimen is the optimal front-line treatment for fit patients with chronic lymphocytic leukemia (CLL). The aim of this study was to evaluate whether a double dose of ofatumumab (O2) combined with FC could improve the CR rate in young (≤65 yrs) and fit patients with CLL. Seventy-eight fit CLL patients from 16 Italian institutions received front-line treatment with the FC-O2 regimen based on the FC schedule (F 25 mg/sqm i.v. d1-3, C 250 mg/sqm i.v. d1-3) combined with 13 doses of O (300 mg i.v. d14; 1000 mg d21 at cycle 1; 1000 mg d1 and d15 at cycles 2-6 and d28 at cycle 6). CLL diagnosis, treatment requirement and response were assessed according to the 2008 iwCLL guidelines. Minimal residual disease (MRD) was evaluated in patients who achieved a complete hematologic response (CR) by flow cytometry in the peripheral blood (PB) and bone marrow (BM), and by RQ-PCR in flow negative cases. The median follow-up of patients was 17 months (range, 5-34), the median age 55.6 years (range, 36.2-65.1), 59.7% patients were IGHV unmutated, 12.1% showed deletion 11q and 10% deletion17p and/or TP53 mutation. The overall response rate was 93.6%, with a CR rate of 62.8% (49 patients). In the 49 patients who achieved a CR, no evidence of MRD in both the PB and BM was observed by flow cytometry in 34 cases (34/49, 69%; 34/78, 43.6%) and by RQ-PCR in 17 (17/49; 34.7%; 17/78, 8.9%). The progression-free survival (PFS) at 24 months was 90.4%. PFS was significantly influenced by the presence of deletion 17p and/or TP53 mutation (68.6% *vs* 94.4%; p=.0006) and by the achievement of CR (95.2 *vs* 83.2%; p=.03). All 17 patients with molecular CR are disease-free at 24 months. The overall survival (OS) at 24 months was 94%. The presence of a TP53 disruption was the only factor with a significant effect on survival (66.7% *vs* 96.4%; p<.0001). Grade 3-4 granulocytopenia was observed in 34.6% of patients and grade ≥3 infections in 9%. Nine (11.5%) patients experienced grade >2 infusion-related reactions during ofatumumab. No treatment-related deaths were recorded. The results of this front-line study demonstrate that the FC regimen combined with an increased dose of ofatumumab shows acceptable toxicity and is associated with a high rate of MRD-negative CRs. These data suggest that the FC-O2 combination is an effective front-line treatment approach for young and fit patients without TP53 aberrations.

B012

FREQUENCY AND CLINICAL RELEVANCE OF PNH CLONES IN A POPULATION OF LOW-RISK AND INTERMEDIATE-1 RISK MDS PATIENTS: A GROM-L STUDY

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Paroxysmal nocturnal hemoglobinuria (PNH) is a acquired non-malignant stem cell disease characterized by the expansion of a population of glycosyl phosphatidylinositol anchor protein-deficient cells, leading to chronic, uncontrolled complement activation leading to intravascular hemolysis and an inflammatory prothrombotic state. PNH is clinically heterogeneous due to the relationship with bone marrow failure syndromes like aplastic anemia and myelodysplastic syndromes (MDS). The detection of a PNH clone in the latter disease may suggest a common immunomediated physiopathology and a better response to immunosuppressive therapy. The aim of the study was to evaluate by high sensitivity multiparametric flow cytometry (MFC) the frequency of PNH clone in patients with a confirmed diagnosis of IPSS low- and Intermediate-1 MDS. From April 2014 to March 2017, 130 peripheral blood (PB) samples have been collected at diagnosis, in 11 Hematological Centers (5 University hospitals and 6 community-based hospitals) located in Rome and in the Latium region. The most commonly used markers were CD59 for red blood cells (RBC), CD24, CD66b, Fluorescent Aerolysin (FLAER) and CD157 for granulocytes (PMN), CD14, FLAER and CD157 for monocytes. The most common gating strategy was based upon morphological parameters, Glycophorin-A and/or CD45 for detecting RBC, CD45, CD33 and CD15 for PMN, CD45, CD33 and CD64 for monocytes. Clone size was assessed by enumerating the percentage of GPI-negative PMN. The target sensitivity was 0.1% and a cluster of 30 events was required to define the clone. Median age was 69 years (range 25-94), 27 pts were RAEB1 (21%), 27 pts RA (21%), 61 pts were RCMD (46%), 4 pts were RARS (3%), 2 pts were 5q- Syndromes (2%), 9 pts were RCUD (7%). With a sensitivity of 0.1%, 8/130 clones (6%) were detected. Seven out of 8 clones were represented by PNH3 cells, with 1 case showing a PNH2 clone. Median size of the clones was 3.9 (range 0.1-36.7). No thrombotic events were recorded. No statistical correlation between the clone and LDH or PMN, Hb or PLT counts was observed. To our knowledge this is one of the largest series of MDS patients prospectively screened by high sensitivity MFC for the presence of a PNH clone. However, the frequency of PNH clones in this category of patients is low and the size of the clones is small. Possible prognostic or therapeutic implication of these observation deserves further observation and a longer follow-up.

ORAL COMMUNICATIONS

Acute Leukemias 1

C001

GENOMIC ANALYSIS OF PML/RARA BREAKPOINTS IN PAIRED DIAGNOSIS/RELAPSE SAMPLES OF PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA TREATED WITH ALL-TRANS RETINOIC ACID AND CHEMOTHERAPY

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It has been reported that t(15;17) occurring in therapy-related Acute Promylocytic Leukemia (t-APL) is frequently generated by cytotoxic drugs targeting the topoisomerase II enzyme. Among these, mitoxantrone (MTZ) has been shown to induce DNA breakage that are frequently located in a "hotspot" region of 8-bp within PML intron 6 in t-APLs developing after breast cancer. The presence of this hotspot in PML has been further confirmed in t-APL occurring in patients who received MTZ for the treatment of multiple sclerosis. Because standard front-line treatment of APL commonly includes anthracyclines and/or MTZ, we hypothesized that disease recurrence in APL may in some cases be therapy-related, rather than being a true relapse of the original leukemia. While this may occur with no change in the PML/RARA isoform, in case of therapy-related disease a different genomic breakpoint location could be detectable in PML or RARA genes at the time of "relapse", possibly with breakpoint been located in one of the "hotspots" PML or RARA genes described in t-APL. We analysed the genomic breakpoints in PML or RARA genes in paired samples of 36 APL patients who relapsed after standard all-trans retinoic acid (ATRA) and chemotherapy. In these samples, we verified the potential involvement of the PML or RARA gene "hotspots" typical of t-APL, and/or changes in DNA breakpoints in relapsed samples. Main patient clinical and biological features are shown in Table 1. As to PML gene analysis, 11 patients had breakpoints in intron 6, 3 in exon 6 and 22 patients in intron 3 (Table 1).

Table 1.

UPN	Age/Sex	Sanz Risk*	Treatment	Time of CR (months)	PML/RARA isoform	PML breakpoint	RARA breakpoint
1	18/F	High	AIDA 2000[#]	43	bcr1	1458-60	12324-6
2	42/F	High	AIDA 2000[#]	9	bcr3	793-4	2285-86
3	42/F	Low	AIDA 2000[#]	12	bcr3	1050-52	8330-32
4	18/F	Intermediate	AIDA 2000[#]	45	bcr3	912 "A" ins	8889
5	46/M	High	AIDA 2000[#]	26	bcr3	971-2	5394-6
6	30/M	Intermediate	AIDA 2000[#]	8	bcr3	930-33	8064-67
7	68/M	High	AIDA 2000[#]	5	bcr3	722	1471
8	56/M	Low	AIDA 2000[#]	14	bcr3	379 "GA" ins	9295
9	29/F	Intermediate	AIDA 2000[#]	NA	bcr1	1356-58	12619-21
10	50/M	Intermediate	LPA 2005[#]	13	bcr1	1977-79	14447-49
11	55/M	Intermediate	LPA99[#]	32	bcr1	1594-96	16272-74
12	31/M	Intermediate	LPA99[#]	45	bcr3	969-91	12158-60
13	34/F	High	LPA99[#]	19	bcr3	951	16477
14	39/M	High	IC-APL[§]	45	bcr3	579	6699
15	29/M	High	IC-APL[§]	19	bcr3	1285-90	1971-76
16	38/M	High	IC-APL[§]	12	bcr3	724	13646
17	NA	NA	MRC[°]	41	bcr3	855	879
18	NA	NA	MRC[°]	10	bcr3	819	13299
19	NA	NA	MRC[°]	12	bcr3	1119-24	14533-37
20	NA	NA	MRC[°]	14	bcr2	892	14040
21	NA	NA	MRC[°]	25	bcr2	661	7676
22	18/F	Intermediate	AIDA 2000[#]	10	bcr2	758-62	12102-06
23	9/F	High	ICC-APL01[°§]	5	bcr1	1285	13157
24	5/M	NA	AIDA 2000[#]	20	bcr3	510	642
25	14/M	High	AIDA 2000[#]	18	bcr3	1323-26	8201-3
26	8/F	High	ICC-APL01[°§]	6	bcr3	1334-5	6897-8
27	10/F	NA	AIDA 2000[#]	105	bcr1	1162-5	9932-5
28	10/F	NA	AIDA 2000[#]	42	bcr3	426-28	451-453
29	77/M	Intermediate	aAIDA[°]	17	bcr1	1723	4409
30	31/M	Intermediate	AIDA 2000[#]	12	bcr1	1378-80	9161-63
31	45/M	Low	AIDA 2000[#]	21	bcr1	1649	3341
32	58/F	High	AIDA 2000[#]	19	bcr3	1209-12	7328-31
33	75/M	Intermediate	AIDA 2000[#]	13	bcr3	1309 "GC" ins	13274
34	44/M	Intermediate	AIDA 2000[#]	8	bcr1	1488-89	12038-39
35	68/F	Intermediate	AIDA 2000[#]	62	bcr3	1033-4	8033-4
36	39/F	Intermediate	AIDA 2000[#]	10	bcr1	1448 "GAAGG" ins	2497

UPN: unique patient number; CR: complete remission; bcr: breakpoint cluster region; NA: not available; ins: insertion.

[*] Sanz and Lo-Coco, 2011
[°] Rego et al, 2013
[§] Testi et al, 2014
[#] Mandelli et al, 2003

PML breakpoints in intron 6 were located between nucleotide positions 1162-1979. In one case (UPN 34) the breakpoint was localized within the hotspot region at position 1482-1489, previously identified in t-APL after MTZ treatment. Breakpoints within PML exon 6 were localized between nucleotides 661 and 892, whereas in PML intron 3 the breakpoints were located between nucleotides 379 and 1335. Concerning the RARA gene, breakpoints were scattered between nucleotides 451 and 16477 with no particular clustering. In all cases, we observed identical genomic breakpoint locations in PML and RARA genes comparing paired diagnostic and relapse samples. Although all APL patients in the present study received topo-II inhibitors MTZ and/or anthracyclines as part of their APL therapy, genomic breakpoints in either PML or RARA at relapse were identical to those present at diagnosis, against our original hypothesis.

C002

MONOCYTES ARE REQUIRED FOR BOTH OPTIMAL ANTI-LEUKEMIC EFFICACY AND RELATED TOXICITIES BY CAR-T CELLS

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Chimeric antigen-receptor (CAR)-T cells promise to cure leukemias relapsed after or refractory to standard treatments. Before this promise is fulfilled, however, a number of crucial issues needs to be solved, namely how to manage toxicities (*e.g.* cytokine release syndrome, CRS; neurotoxicities) and how to circumvent the emergence of secondary resistance (*e.g.* those due to target-antigen loss). Unfortunately, these issues cannot be addressed pre-clinically in currently available NSG mouse models because they lack human hematopoiesis and develop xenograft-*versus*-host disease (X-GVHD), preventing the evaluation of long-term effects. We have developed an innovative xenotolerant model by transplanting human HSCs intraliver in newborn NSG mice transgenic for human SCF, GM-SCF and IL-3 (SGM3). Differently from NSG, SGM3 mice reconstituted high levels of human T cells, which, once transferred in secondary recipients, persisted up to 200d without causing X-GVHD. We therefore designed secondary transfer experiments in leukemic and/or HSC-humanized SGM3 mice for properly studying CAR-T cell efficacy and associated toxicities. SGM3-derived T cells were transduced *ex vivo* with either a CD19 or a CD44v6 CAR. Once transferred in recipients engrafted with CD19+/CD44v6+ leukemia, CD19 or CD44v6 CAR-T cells equally mediated tumor clearance in the absence of malaise or increase in hIL-6. However, 200d after CAR-T cell infusion 50% of responding mice relapsed despite CAR-T cell persistence *in vivo*. Relapses were mostly characterized by down-regulation of CD44v6 membrane expression or CD19 epitope loss, respectively. Conversely, transfer of SGM3-derived CAR-T cells in leukemic SGM3 mice that had been previously humanized with HSCs resulted in the development of a clinical syndrome that phenocopied the CRS (high fevers and elevated IL-6). Strikingly, mice recovering from CRS benefited from durable remission. Tocilizumab administration at the time of CAR-T cell infusion efficiently prevented CRS without interfering with anti-leukemic effects. Tocilizumab was not able to restrain neurotoxicities, while prophylactic anakinra administration prevented both the CRS and neurotoxicities. To conclude, monocytes are required for both optimal anti-leukemic efficacy and the occurrence of CRS and neurotoxicities; tocilizumab does not interfere with anti-leukemic activity of CAR-T cells but is poorly effective in preventing neurotoxicities, while anakinra prevents CRS and neurotoxicities.

C003

CONTRIBUTION OF MINIMAL RESIDUAL DISEASE EXPLAINS THE HETEROGENEOUS OUTCOME OF NUCLEOPHOSMIN-1 MUTATED ADULT ACUTE MYELOID LEUKEMIA

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NPM1 mutations identify a subset of patients with Acute Myeloid Leukemia (AML), who have a favorable course of disease. However, recent evidences suggest that the persistence of minimal residual disease (MRD), as determined by quantifying residual NPM1 mutated copies, can revert the NPM1 favorable outlook into an adverse course of disease. Based on this, the aim of our study was to investigate if there is a role in terms of prognostic impact for flow cytometry measured MRD (FC-MRD) in patients with NPM1mut AML and to compare with a population of patients with NPM1wt AML. We analyzed 69 patients with NPM1mut AML who were in complete remission (CR) after intensive induction of EORTC-GIMEMA protocols. Twenty out of 65 pts (31%) carried a concomitant FLT3-ITD and 51/66 (77%) had a normal karyotype. After consolidation, a number of residual leukemic cells (RLCs) ≥3.5x10(-4) (0.035%) in the bone marrow was regarded as a condition of MRD positivity. Among the 69 NPM1mut patients, the rate of FC-MRD negative CR was significantly lower (5/69, 7%) than among NPM1wt (39/134, 29%) (p<0.001). Although there was not a statistically significant difference, FC-MRD negative/NPM1mut pts had a lower Cumulative Incidence of Relapse (CIR) than those FC-MRD negative/NPM1wt (25% *vs* 60%). We also evaluated the impact of autologous (AuSCT) or allogeneic (ASCT) transplant on the outcome of FC-MRD positive/NPM1mut pts. The overall survival (OS) was significantly longer for pts submitted to ASCT (no=14) than those (no=15) who received AuSCT (93% *vs* 33%, p=0.011). This was also confirmed by excluding from the analysis FLT3-ITD pts. The adjusted analysis for competing variables, showed that the type of transplant (ASCT *vs* AuSCT) was the only significant variable affecting OS and DFS (p=0.001 and 0.003, respectively). In conclusion, although qRT-PCR represents the gold standard platform for MRD detection in NPM1mut pts, FC-MRD is feasible and confirms that the quality of CR is critical to discriminate pts with different outcome. In fact, NPM1mut pts have few chances to reach a FC-MRD negative CR, and, in case of MRD positivity, the adverse outcome can be substantially reverted by the timely use of ASCT.

C004

CRITICAL ROLE OF NOTCH IN B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: B-ALL is the leading cause of cancer-related death in children. There is still a need of more efficient therapies for the subset of refractory patients. Our group has previously shown that Notch-3 and Notch-4 promote human B-ALL cell survival in presence of stromal cell support. However, the contribution of Notch signalling *in vitro* and *in vivo* to chemotherapy in B-ALL has not yet been investigated. *Aims:* In this study, we used B-ALL cells to analyse the contribution of Notch signalling to B-ALL pathogenesis in terms of prognosis, proliferation survival and drug response *in vitro* and in mice xenograft models of B-ALL. *Methods:* Flow cytometry and western immunoblotting were used to study the expression of Notch receptors and ligands. Drugs used were Cytarabine (Ara-C), Dexamethasone (Dexa) and Doxorubicin (Doxo) alone or in combination with Notch modulators including anti-Notch blocking antibodies, gamma secretase inhibitors (GSIs), and Notch transcription factor inhibitor (SAHM1). Mice xenograft model of B-ALL were obtained by injecting the B-ALL line RS4;11 in NOD/Shi-scid/IL-2R null mice (NOG). *Results:* Western blot and flow cytometric analysis showed that B-ALL cell lines as well as primary blast cells displayed the same Notch expression pattern consisting in high expression levels of Notch1, Notch3, Notch4, Jagged2, DLL3 and DLL4. Notably, in primary blast cells deriving from patients the expression of Notch3, Notch4, Jagged2, DLL3 and DLL4 was significantly higher in the cases refractory to treatment as compared to patients achieving complete remission, thus suggesting that Notch signalling could be involved in the response to chemotherapy. In line with this hypothesis, we found that the treatment *in vitro* of B-ALL cell lines with Ara-C or Dexa down regulates the expression of Notch receptors. In addition, Notch inhibitors significantly improved *in vitro* the cytotoxicity of Ara-C, Dexa and Doxo towards B-ALL cell lines. Finally, we observed that the administration to mice of a pan Notch inhibitor, *i.e.* the GSI XII, significantly lowered the CD19+ leukemic burden in the bone marrow of recipient mice, potentiating anti leukemic effect of ARA-C. *Conclusions:* In this study, we used both *in vitro* and *in vivo* assays to highlight the critical role of Notch signalling in B-ALL cell response to chemotherapy, revealing that Notch signalling is a possible therapeutic strategy to eradicate minimal residual disease in B-ALL.

C005

DECITABINE IN PATIENTS WITH NEWLY DIAGNOSED AND RELAPSED ACUTE MYELOID LEUKEMIA: THE REAL LIFE EXPERIENCE FROM THE "ITALIAN TRIVENETO REGISTRY"

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Background: The hypomethylating agent Decitabine (DAC) has emerged as an alternative to first line and salvage therapy in acute myeloid leukemia (AML), particularly in elderly patients (pts) which may not benefit from intensive chemotherapy (CHT). However, outside of clinical trials, no data are available regarding efficacy and safety of DAC in clinical practice. *Patients and Methods:* We performed a preliminary analysis from a large cohort of 82 AML pts treated with DAC in 8 Italian Hematological Centers (Udine, Padua, Trieste, Verona, Vicenza, Pordenone, Treviso, Aviano) from February 2015 to March 2017 (the recruitment of cases is still open). Fifty-six (68%) pts received DAC as first line treatment (Group 1) and 26 pts as salvage therapy (Group 2). Median age was 73 yrs (75 in Group 1 and 68 in Group 2) and the PS was less than 60% (Karnofsky) in 20/82 cases (24%). In the Group 2, 14/26 (54%) pts were treated after conventional CHT, 5/26 after Allo-SCT and 7/26 after azacitidine therapy. The median baseline BM blast percentage was 50%(range 20-95) in the Group 1 and 30%(10-100) in the Group 2(P=ns). The standard schedule of DAC was 20 mg/mq daily for 5 days-every 4 weeks, in all cases. *Results:* A total of 345 DAC cycles were given to the 82 pts with a mean of 5±4,5 cycles in Group 1 and 2,5±1,8 in Group 2 (P=0,04); 32/84 pts received >4 cycles. The Overall Response Rate (ORR=CR+PR) was 34%, significantly higher in Group 1(45%) compared to Group 2(13%), P=0,008. The median follow up was 4,5 months (range 1-22). At last follow-up 50 pts(61%) are still alive and 32(39%) are dead. The main cause of death was disease progression (69%). The most common toxicities were myelosuppression and documented infectious complications. The median OS from the start of DAC therapy was 12,3 mths for the whole population whit a significant OS advantage in Group 1 (median OS 13 mths *vs* 6 mths-P=0,007; Figure 1).

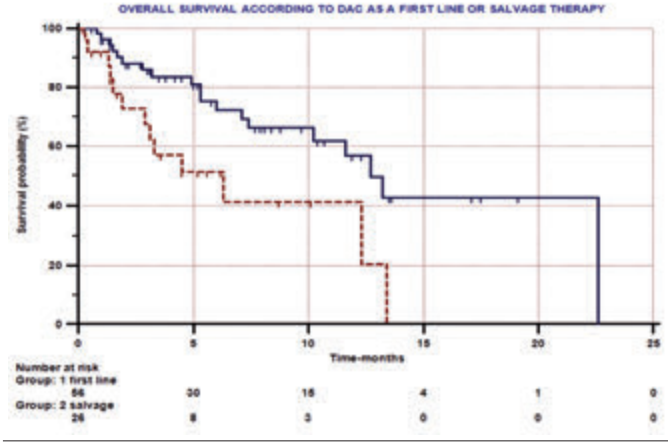

Figure 1.

Univariate and Multivariate analysis of factors affecting DAC response and OS are ongoing (pts recruitment still open). *Conclusions:* These data show the efficacy and the emerging role of DAC in the real life management of AML, with a significant better performance in first line therapy, even in elderly and unfit pts unsuitable for intensive CHT (ORR 45%, median OS 13 mths). The efficacy of DAC, as a single agent for salvage therapy, may probably be improved with combined treatment strategies and/or with different DAC schedules that increase its anti-leukemic effect.

Infections

C006

PROSPECTIVE STUDY COMPARING NEUTROPENIC ENTEROCOLITIS IN 147 LYMPHOMA PATIENTS TRANSPLANTED WITH BEAM VS FEAM: ROLE OF ULTRASONUND ON INCIDENCE, DIAGNOSIS, TREATMENT AND OUTCOME IN THIS LIFE THREATENING COMPLICATION

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High-dose chemotherapy with peripheral blood progenitor cell (PBPC) collection followed by a myeloablative conditioning and autologous stem cell transplantation (ASCT) is considered the standard of care of relapsed/refractory non Hodgkin/Hodgkin lymphoma (NHL/HL). Widely adopted conditioning regimens are BEAM and FEAM. Neutropenic enterocolitis (NEC) is a life threatening complication of patients (pts) treated with chemotherapy (CHT) with mortality rate up to 50%. NEC is a clinical syndrome in neutropenic patients (pts) characterized by abdominal pain (AP), fever (F) and diarrhoea (D). Ultrasound (US) was used to evaluate bowel-wall thickening (BWT), and > 4 mm is considered diagnostic of NEC. Early diagnosis is crucial to start treatment and reduce mortality. Objective: 1. evaluate if NEC incidence and outcome differs in BEAM *vs* FEAM and 2. evaluate prospectively if Bed-side-US(BUS) can detect early signs of NEC and guide a prompt treatment (conservative or surgical) in order to reduce mortality. *Methods:* in the last 5 years all pts with NHL/HL admitted in Our BMT Unit wards at University of Pisa (Italy), undergoing ASCT were prospectively enrolled. Abdominal US was performed, baseline before treatment, and as only one symptom (or a combination) appeared within 12h from onset: F and/or D and/or AP in CHT-related neutropenic pts. *Results:* 95 pts were conditioned with BEAM and 52 pts with FEAM. NEC was diagnosed in N=19/52 FEAM and in N=25/95 BEAM patients. Incidence was 36% and 25% respectively, without a statistically significant difference (P=0.234). Two pts died/19 in FEAM arm (10.5%) and 2pts/24 in BEAM arm (8.3%), without a statistically significant difference (P=0.778). At Dx, F was absent in 18/44 NEC episodes (40%). All pts were treated promptly as BUS allowed diagnosis conservatively except one 1 pts who underwent surgery. *Conclusions:* BUS allowed to detect early signs of NEC and to start prompt treatment in this life threatening complication, of NHL/HL pts undergoing ASCT. This is a prospective study thus the true incidence of NEC in NHL/HL undergoing ASCT should not be underestimated. There is not a statistically significant difference in incidence and outcome of NEC in pts conditioned with BEAM in respect to FEAM. Fever is not a conditio sine qua non for NEC diagnosis. A low mortality rate in pts with a 25% to 36% chance of developing NEC suggests that a prompt BUS in neutropenic patients does reduce mortality.

C007

GRANULOCYTE TRANSFUSIONS AT APPROPRIATE DOSES IMPROVE OUTCOME IN BACTERIAL INFECTIONS IN NEUTROPENIC PATIENTS WITH HEMATOLOGICAL DISEASES

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Granulocyte transfusions (GTs) are used to booster antimicrobial drugs in severely neutropenic hematological patients (pts). However the optimal dose and the efficacy in clinical practice are still debated, as well as the setting of infections that could improve after them. We retrospectively evaluated the infection-related mortality (IRM, defined as death from infection within 30 days after the last GT) in 57 consecutive pts (median age 45.5 years, range 20-74; M/F 39/18) with hematological malignancies (48 AML, 5 NHL, 3 ALL, 1 SMD) receiving at least one GT. The indications for GTs were presence of absolute neutrophil count (ANC)<0.5x10^9/l, fever with evidence of bacterial infections and unresponsiveness to appropriate antimicrobial therapies for at least 48 hours. The study was registered at www.clinicaltrials.gov

(NCT022544230). Infections were mono-microbial (42 cases) or poly-microbial (15 cases); the most frequent pathogens were Klebsiella pneumoniae (14), Escherichia coli (9) and Pseudomonas aeruginosa (7). Sepsis occurred in 54 pts. On average, 4 GTs (1-14) per patient were transfused. The median granulocyte dose per transfusion was $1.95x10^8$/kg (0.53-7.63). The IRM was 35% (20/57). Pts were grouped according to the median doses of granulocytes received. Doses were derived from the current Italian transfusion regulation and included low-doses (<$1.5\text{-}x10^8$ cells/Kg), standard-doses ($1.5\text{-}3.0x10^8$ cells/Kg) and high-doses (>$3.0x10^8$ cells/Kg). Twelve pts received low-doses, 28 standard-doses and 17 high-doses. The IRM was not influenced by the number of GTs or by the total amount of granulocytes received, whereas a dose-related effect was observed. Actually, receiving median doses lower than 1.5 or greater than $3.0x10^8$/Kg was associated with higher mortality both at univariate (p=0.002) and multivariate analysis (OR 18.4, 95%CI 2.9-114.6, p=0.002) (Table1). Moreover, median survivals significantly differed in according to different doses received (27 days *vs* 61 days, in standard- and nonstandard-groups respectively; HR 4.0, 95%IC 1.6-10.1; p=0.002). Finally, pts receiving GTs at doses lower or greater than standard had increased risk for subsequent ICU admission (p=0.024). These findings suggest that appropriate GT doses can improve the post infection survival of severely neutropenic hematological patients. Transfusion-related immunomodulation or leukostasis may underlie the detrimental effect of high dose GTs and deserve further investigations.

Table 1. Clinical and transfusion findings in 57 bacterial infections.

Characteristics		p*	p§
Sex Number of pts (IRM%)	Male: 39 (38.4) Female: 18 (27.7)	0.555	0.077
Underlying disease Number of pts (IRM%)	Myeloid neoplasms: 49 (36.7) Lymphoid neoplasms: 8 (25.0)	0.699	0.522
Age over 60 years Number of pts(IRM%)	Yes: 12 (50.0) No: 45 (31.1)	0.309	0.101
Chemotherapy lines Number of pts (IRM%)	First line 38 (36.8) Subsequent lines: 19 (31.5)	0.775	0.966
ICU admission Number of pts (IRM%)	Yes: 19 (78.9) No: 38 (13.1)	**<0.001**	-
Allo-HSCT Number of pts (IRM%)	Yes: 15 (40.0) No: 42 (33.3)	0.755	0.512
Blood stream infection Number of pts (IRM%)	Yes: 54 (37.0) No: 3 (0)	0.545	0.999
Pneumonia Number of pts (IRM%)	Yes: 4 (50.0) No: 53 (33.9)	0.607	0.999
XDR infection Number of pts (IRM%)	Yes: 24 (41.6) No: 33 (30.3)	0.411	0.232
Median PMN dose $1.5\text{-}3.0x10^8$/kg Number of pts (IRM%)	Yes: 28 (14.2) No: 29 (55.1)	**0.002**	**0.002**
Age, years, median value (range)	Live: 44 (35-74) Deaths: 55 (22-71)	0.067	0.221
Days of neutropenia, median value (range)	Live: 17 (6-65) Deaths: 15.5 (3-66)	0.940	0.368
GTs per course, median value (range)	Live: 3 (2-6) Deaths: 3.5 (1-14)	0.390	0.558
Number of transfusions/days of neutropenia, °median value (range)	Live: 16.6 (5.3-57.1) Deaths: 20.8 (3.2-100)	0.375	0.930

On the whole, 20 deaths were recorded among bacterial infections. IRM: infection-related mortality; ICU: Intensive care Unit; Allo-HSCT: allogeneic hematopoietic stem cell transplantation; XDR: extensively drug resistant; GTs: granulocyte transfusions.
*univariate analysis; §multivariate analysis

C008

IDENTIFICATION AND VALIDATION OF DIAGNOSTIC CUT-OFF VALUES FOR THE DIAGNOSIS OF INVASIVE ASPERGILLOSIS BY ELISPOT ASSAY IN HIGH RISK PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Early diagnosis is crucial for outcome of invasive aspergillosis (IA), but difficult due to the lack of non-invasive gold standard diagnostic tests. Recently, the identification of fungal-specific immune responses was proposed as alternative diagnostic marker of invasive mould diseases. To identify the best diagnostic cut-off value for Aspergillus-specific Elispot assay, we initially collected from different centers, 523 PB samples from 20 patients with proven IA and 186 control patients. We measured the frequencies of Aspergillus-specific T-cells secreting IFNg and IL10 after stimulation with germinated, heat-inactivated conidia of Aspergillus spp. We used STATA software to determine ROC curve per patients analysis. We subsequently validated the identified cut-off in a monocenter prospective cohort of 100 consecutive hematologic patients, mainly with acute leukemia, during either induction or salvage treatment. We carried out Elispot assay on 277 PBMC samples obtained from 1 proven, 6 probable, 7 possible IA and 86 control patients. Sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), likelihood ratios positive and negative (LR+/LR-) and the efficiency of the Aspergillus-specific Elispot assay to diagnose IA were calculated with a confidence interval (CI) of 95%. ROC curve elaboration using data related to the first patients' cohort showed the presence of at least 40 Spot Forming Cells (SFC)/10^6 PBMC for IL10-secreting T cells as the best diagnostic cut-off value, with SE=89.5% SP=88.6% PPV=45.9% NPV=98.7% LR+=7.8289 LR-=0.1188. The presence of 1 positive sample in a per patient analysis comparing proven and probable IA *versus* controls of prospective cohort showed SE=71.4% SP=87.2% PPV=31.25% NPV=97.4% LR+=5.584 LR-=0.328. When the documentation of 2 consecutive samples was considered, SP, PPV and NPV reached 95.4%, 55.6%, 97.6%, respectively, with performance improvement due to the reduction of positivity in the control group. Enumeration of Aspergillus-specific IL10-producing T cells could be useful for diagnosis of IA. The derived cut-off should be confirmed in multicenter prospective studies. However, the high NPV and the increasing PPV of two positive consecutive samples suggested that Elispot assay, which is not influenced by concurrent azole prophylaxis, could be used in combination with other non-cultural methods in a diagnostic-driven work-up to improve proper administration of antifungal therapy.

C009

MULTIDRUG-RESISTANT GRAM NEGATIVE BACTEREMIA IN HEMATOLOGIC PATIENTS: INCIDENCE AND INFECTION CONTROL PRACTICES IN A MONOCENTRIC EXPERIENCE

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Prolonged neutropenia and chemotherapy-induced mucositis render hematologic patients highly vulnerable to Gram negative multidrug-resistant bacteria (GNB-MDR), including extended spectrum -lactamase-producing (ESLBLs) Escherichia coli and Klebsiella pneumoniae, carbapenem-resistant Pseudomonas aeruginosa (CRPA) and carbapenem-resistant Enterobacteriaceae (CRE) producing carbapenemases. Klebsiella pneumoniae carbapenemase (KPC) is the most common carbapenem resistance mechanism among Enterobacteriaceae. To evaluate the incidence and clinical outcome of GNB-MDR colonizations/infections and to identify the most important associated factors, we conducted a prospective surveillance program from 2013 to 2016 in hematologic patients (HP). Among 810 HP (mean length of stay, 35 days), 193 bacteremias were observed and GNB were responsible for 68% of sepsis. Out of 132 GNB bacteremia, 65 (49%) were due to E. coli (16% ESBL+) 28 (21%) to Klebsiella spp (25% KPC), 23 (17%) to P. aeruginosa (20% CRPA), 8 (6.5%) to Enterobacter spp, 8 (6.5%) to other species. The incidence rate of CRE bacteremia was 5% (7 KPC bacter-

aemia); all patients were undergoing to intensive chemotherapy and 43% had severe neutropenia. KPC bacteremia was recorded after a median of 15 days of hospital stay, with a mortality rate of 57%. After the last KPC sepsis case, antimicrobial prophylaxis with fluoroquinolones was discontinued in all patients and a progressive decrease of CRE colonization and sepsis was documented. No death due to other GNB-MDR bacteremia was documented. CRE colonizations from rectal swabs were 43 (5.3%): KPC type 37 (11 in 2013, 18 in 2014, 3 in 2015, 5 in 2016) VIM type 6 (2 in 2014, 4 in 2015). Recently the emergence of antibiotic resistance has become a concern and the epidemic spread of resistant Enterobacteriaceae may influence strategies toward the use of quinolone prophylaxis. Consequently local epidemiological trend must be taken into account when deciding for quinolone prophylaxis. In our experience the only CRE responsible for sepsis was K. pneumoniae, on the contrary to other experiences in which infections were due to different CRE as E. cloacae, E. coli, K. oxytoca. In conclusion considering the high morbidity and mortality-rate associated to GNB-MDR infection, particularly to CRE–KPC, strict routine surveillance, screening for carriage, antimicrobial stewardship and new antimicrobial agents, as ceftolozane/tazobactam and ceftazidime/avibactam, are needed.

C010

TENOFOVIR *VERSUS* LAMIVUDINE FOR PREVENTION OF HEPATITIS B VIRUS REACTIVATION AMONG PATIENTS WITH AGGRESSIVE LYMPHOMAS UNDERGOING FRONT-LINE ANTHRACYCLINE-CONTAINING CHEMOTHERAPY: A SINGLE-CENTER, REAL-LIFE EXPERIENCE

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Primary antiviral prophylaxis (PAP) is essential for patients seropositive for hepatitis B surface antigen (HBsAg) who undergo intensive cytotoxic chemotherapy for lymphoma. Lamivudine (LAM) is the most commonly used drug, but alternative nucleos(t)ide analogs such as entecavir and tenofovir disoproxil fumarate (TDF) may provide a better efficacy. We compared the efficacy and safety of LAM (systematically administered from July 2004 to June 2009; LAM-cohort) and TDF (systematically administered from February 2009 to June 2015; TDF-group) in preventing HBV reactivation in HBsAg-positive patients with advanced-stage diffuse large B cell (DLBC) non-Hodgkin lymphoma (NHL) or classic Hodgkin lymphoma (HL). All patientswere scheduled to receive full-course of anthracycline-containing chemotherapy, according to R-CHOP or ABVD regimen. During the study period, of the 60 HBsAg-positive patients who received PAP with either LAM or TDF, 20 were excluded due to liver dysfunction and/or high serum HBV-DNA levels at baseline, chemotherapy reduction or delay for acute toxicity not due to HBV infection, or because lost to follow-up. Twenty-one patients received LAM and 19 received TDF. The rate was significantly lower in the TDF-group compared with the LAM-cohort for HBV reactivation (0% *vs* 52.3%, respectively; p=.0002), HBV-related hepatitis (0% *vs* 19%, respectively; p=.04) and chemotherapy disruptions (0% *vs* 23.8%, respectively; p=.02). No difference was observed between the two groups in terms of incidence of PAP-related adverse events (5.2% [TDF-group] *vs* 19% [LAM-cohort]; p=.18). Furthermore, at a median follow-up of 34 months there was no significant difference between the TDF-group and LAM-cohort in terms of progression free survival of underlying lymphoma (94.7% *vs* 84.8%; p=.37). In a multivariate analysis, TDF prophylaxis and HBV-DNA ≤73 IU/mL were associated with a lower risk for HBV reactivation (p values, <.0001 and =.03, respectively). Therefore, TDF should be suggested for front-line prophylaxis in HBsAg-positive patients receiving induction chemotherapy for aggressive lymphomas, to prevent HBV reactivation and evolution in HBV-related hepatitis, which could lead to chemotherapy disruption. Further studies are warranted to confirm these findings.

Chronic Lymphocytic Leukemia

C011

VEMURAFENIB PLUS RITUXIMAB IS A VERY EFFECTIVE CHEMOTHERAPY-FREE REGIMEN IN RELAPSED OR REFRACTORY HAIRY CELL LEUKEMIA

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Background: HCL responds well to purine analogs, but up to 50% of patients eventually relapse. After identifying the BRAF-V600E mutation as the genetic cause of HCL (NEJM 2011), we documented in 26 relapsed/refractory HCL patients a high response rate (96%) with the BRAF inhibitor vemurafenib given for a median of 16 weeks (NEJM 2015). Responses were obtained after a median of 8 weeks and included 35% complete remissions (CR) and 61% partial remissions (PR). In all patients, residual disease could be documented in the bone marrow and the median relapse-free survival was 9 months from the end of treatment. Rituximab has the potential of targeting HCL cells resistant to vemurafenib, and thus of improving clinical outcome in a chemotherapy-free manner. *Methods:* In this ongoing academic phase-2 single-center trial (EudraCT 2014-003046-27), HCL patients refractory to, or relapsed after, purine analogs receive vemurafenib (960 mg b.i.d. orally) for 8 weeks and concomitant rituximab (375 mg/m^2 intravenously) every 2 weeks. Rituximab is also given four times every 2 weeks after the end of vemurafenib. CR is defined as normal blood counts, no splenomegaly and no leukemia visible in the bone marrow biopsy and in the blood through nonimmunological stains. *Results:* We have so far enrolled 29 patients (median age 57 years; range 35-81) in 24 months. Adverse reactions were mostly of grade 1-2, reversible and consistent with the known toxicity profile of the two drugs when used alone. Notably, a CR was achieved by all 21 patients evaluable for efficacy (100%). These included patients previously treated with purine analogs that, in addition, had been refractory to prior rituximab monotherapy (n=3) and/or had relapsed after a prior BRAF inhibitor (n=6, of whom 4 had obtained a short-lived PR and 2 a CR after the BRAF inhibitor). Furthermore, 15/19 evaluable patients (79%) obtained the CR as early as after 4 weeks of vemurafenib and two concomitant rituximab infusions. Minimal residual disease (MRD), as evaluated by allele-specific PCR (limit of detection: 0.05% BRAF-V600E copies), was undetectable in the bone marrow of 14/20 (70%) evaluable patients; in 8/14 patients (57%), MRD clearing was reached even before sequential rituximab dosing post-vemurafenib. At a median follow-up of 11 months (range 1.5-24), progression-free survival was 100%. *Conclusions:* Vemurafenib plus rituximab is a short, safe and very effective non-myelotoxic regimen for relapsed/refractory HCL.

C012

STAT3 ACTIVATION INDUCES NEUTROPHILS' APOPTOSIS IN T-CELL LARGE GRANULAR LYMPHOCYTE LEUKEMIA THROUGH FAS LIGAND SECRETION

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T large granular lymphocytes leukemia (T-LGLL) is a rare disease characterized by the clonal expansion of T-large granular lymphocytes (T-

LGLs), with CD8+/CD4- or less frequently CD4+/CD8dim/- phenotype. Severe neutropenia represents the major disease feature and is related to Fas ligand (FasL) secretion by T-LGL which induces Fas mediated apoptosis of neutrophils. Activation of JAK/STAT signalling represents one of the most important deregulated pathways in T-LGLL, moreover somatic STAT3 mutations conferring constitutive activation has been described in 30-40% of patients. With this as a background, we investigated whether FASL induced neutropenia is related to STAT3 activation. A cohort of 101 patients affected by T-LGLL was studied for the presence of neutropenia (ANC<1,500/mm^3) and STAT3 mutation by Sanger Sequencing and ARMS PCR. By flow, LGL samples were analyzed for CD3, CD4, CD8, CD16 and CD56 expression. FasL mRNA levels were studied by RT-PCR. Patient's PBMC were treated with IL-6 and IL-15 (20ng/ml) and STAT3 inhibitor STATTIC. Among our cohort of patients, a significant association between neutropenia and the presence of STAT3 mutation was found (p<0.0001). Neutropenic and STAT3 mutated patients were characterized by CD3+/CD8+/CD16+/CD56- immunophenotype. By western blot analysis we showed that high STAT3 tyrosine phosphorylation was observed in LGL samples of these patients towards other immunophenotypic subgroups (p<0.01). FAS ligand expression by RT PCR was significantly higher in CD3+/CD8+/CD16+/CD56- patients (p<0.01) and treatment with STATTIC decreased both STAT3 phosphorylation and FAS ligand mRNA expression. Consistently, STAT3 phosphorylation trigger with IL-6 or IL-15 led an increase of FasL transcription levels (1.59- and 2.01-fold respectively). Finally, pre-treatment of patients' PBMC for 1 hour with STATTIC before ILs blocked IL-6 or IL-15 effects preventing STAT3 phosphorylation induction and the increase of Fas ligand transcription. Our data demonstrated a new link between STAT3 mutation/activation and development of neutropenia. We showed that STAT3 is crucial in Fas ligand regulation and we explained the high levels of Fas ligand in CD8+/CD16+/CD56- patients characterized by high STAT3 activation. These data emphasize JAK-STAT inhibition as a new therapeutic target leading to LGL apoptosis and FASL mRNA expression.

C013

THE ROLE OF GENETIC-BASED PROGNOSTIC FACTORS IN PREDICTING MINIMAL RESIDUAL DISEASE NEGATIVITY IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS TREATED WITH FLUDARABINE, CYCLOPHOSPHAMIDE AND OFATUMUMAB

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Fludarabine, cyclophosphamide and rituximab (FCR) is the optimal front-line therapy for fit chronic lymphocytic leukemia (CLL) patients. Minimal residual disease (MRD) is the single best predictor of long-term outcome after FCR, independent of biologic prognostic markers. We aimed at exploring if conventional biologic markers (IGHV mutations/FISH lesions) and TP53, NOTCH1, BIRC3, SF3B1 mutations, assessed at disease progression, can predict the MRD negativity achievement after first-line therapy with FC+ofatumumab (O) (GIMEMA LLC0911 trial). MRD was evaluated in responding patients by 8-color flow cytometry in the peripheral blood (PB) and bone marrow (BM) after 6 FCO cycles (month+8) and every 6 months thereafter; flow MRD- cases were analyzed by RQ-PCR. Eighty young (≤65 yrs) and fit CLL patients from 15 Italian centers were enrolled; 65 responding patients underwent MRD evaluation at month+8. By flow cytometry, 25/65 cases (38%) were MRD+ in the PB and/or BM and 40 (62%) MRD-. The absence of del17p/TP53mut/del11q was associated with the MRD negativity: 37 MRD- (74%)/13 MRD+ in +12/neg FISH/del13q/TP53WT *vs* 2 MRD- (14%)/12 MRD+ in del17p/TP53mut/del11q (p=0.0001). By integrating FISH and gene mutations (Rossi *et al.*, 2013), the high (n=6) and intermediate (n=22) risk groups (del17p/TP53/BIRC3+ or del11q/NOTCH1/SF3B1+) showed a significantly lower proportion of MRD- cases (36%, 10/28) than the low (n=21) and very-low (n=15) risk groups (+12/neg FISH/del13q/WT for 4 genes: 81%, 29/36) (p=0.0003). The 40 flow MRD- cases were evaluated by RQ-PCR: 22 were reclassified as MRD+. Accordingly, 47/65 cases (72%) were MRD+ and 18/65 (28%) MDR- at the end of FCO. Mutated (M)-IGHV was significantly associated to a molecular MRD- (12 MRD-/15 MRD+, 44%) compared to unmutated (UM)-IGHV (5 MRD-/32 MRD+, 13%) (p=0.0092). When M-IGHV status is reinforced by the absence of del17p/TP53mut/del11q, the association with a MRD negativity got stronger (p=0.0036). A multivariate model including FISH lesions, gene and IGHV mutations supports the independent role of FISH and IGHV in predicting MRD negativity by flow and RQ-PCR, respectively (Table 1). In conclusion, in CLL patients after FCO, MRD negativity by flow (62%) can be predicted by the FISH profile. A deeper MRD negativity by RQ-PCR (28%) can be anticipated by the IGHV status or by combining IGHV and FISH. A longer follow-up will determine if these parameters can identify patients who maintain over time a good quality of response.

Table 1. Biologic features of the 65 CLL patients evaluated for MRD.

Median age	59 years (36-65)
Morphology (tipycal/atypical)	31/7
IGHV (unmutated/mutated)	37/27
CD38 (pos/neg; cut-off 20%)	35/30 (54%)
CD49d (pos/neg; cut-off 30%)	37/23 (62%)
ZAP70 (pos/neg; cut-off 20%)	9/50 (15%)
FISH: del17p	2/64 (3%)
del11q	11/64 (17%)
trisomy 12	5/64 (8%)
del13q	21/64 (33%)
No FISH lesions	25/64 (39%)
***TP53* mutation** (one TP53mut+del17p+)	2/65 (3%)
***NOTCH1* mutation**	9/65 (14%)
***BIRC3* mutation**	3/65 (5%)
***SF3B1* mutation**	9/65 (14%)

C014

WHOLE-EXOME SEQUENCING REVEALED NO RECURRENT MUTATIONS WITHIN THE PI3K PATHWAY IN RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS PROGRESSING UNDER IDELALISIB TREATMENT

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Introduction: Idelalisib (IDELA)a inhibits phosphatidylinositol 3-kinase delta (PI3K) and is approved for the treatment, in combination with rituximab (R), of patients with relapsed chronic lymphocytic leukemia (CLL). In randomized, controlled trials, IDELA+R showed high response rates with improved PFS and OS as compared to placebo+R. Disease progression after response occurs, indicating that escape mechanisms may develop. The molecular basis for relapse or progressive disease (PD) in CLL patients treated with IDELA has not been characterized. *Methods:* Peripheral blood mononuclear cells (PBMCs) were collected from 13 CLL patients enrolled in phase 3 studies 116 (IDELA+R *vs* placebo+R); 116 extension and 119 (IDELA+ofatumumab). Sample selection criteria were: treatment of at least 180 days (range:243–703), achieving at least partial nodal response followed by PD, PD not occurring during drug interruption, PD not associated with Richter's transformation, and availability of PBMC samples at baseline and time of PD. Whole-exome sequencing (WES) was conducted on

matched samples from 13 subjects. In 6/13 cases, DNA was available from CD19+/CD5+ enriched tumor cells, and neutrophils or T-lymphocytes served as a source of germline DNA. These 6 patients were considered as a discovery set for mutational analysis. Established bioinformatics tools were used for detection of somatic mutations and for the comparison of baseline and PD samples. *Results:* Baseline clinical patient profiles indicated that 12/13 patients with PD had unmutated IGHV genes and 8 patients carried TP53 aberrations (del(17P); TP53 mutations). WES resulted in a mean read depth of 106X within the targeted coding region. In the discovery set, on average, 25 somatic mutations (range: 4–44) at baseline and 32 mutations (range: 15–81) at progression were identified. By comparing baseline and PD samples, we identified 88 PD-associated mutations. These specific mutations were tested for in a set of 13 patient samples and no progression-associated mutations were identified in more than 1 patient. In particular, no progression-associated mutations were identified in the PI3K pathway or in any other related pathway. *Conclusions:* This analysis detected no relapse-associated mutations in these patients. No mutations were identified in the drug-binding site or in any other related signaling pathway. Based on these results, there is no common mutational mechanism of IDELA resistance in this patient group.

C015

ALLOGENEIC STEM CELL TRANSPLANTATION *VERSUS* B-CELL-RECEPTOR INHIBITORS IN 17P DELETION AND/OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA: A RETROSPECTIVE COMPARATIVE ANALYSIS OF 'REAL LIFE' APPROACHES TO HIGH RISK PATIENTS

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Allogeneic stem cell transplantation (alloSCT) has been indicated in chronic lymphocytic leukemia (CLL) with 17p deletion (del) and in early relapsed/refractory patients. B-cell receptor inhibitors (BCRi) have shown high efficacy with low toxicity, making the choice of alloSCT challenging. The aim of the study is to highlight the outcome of clinical approaches available in the era of the new drugs in high risk CLL patients. This is a multicenter retrospective analysis on 144 patients. Inclusion criteria were: i) age ≤70 years and no comorbidities unacceptable for alloSCT ii) responding to one of the EBMT criteria for elegibility to alloSCT in CLL iii) alloSCT from 2001. Patients were assigned to alloSCT or BCRi based on physician and/or patient choice or unavailability of a donor. The analysis started from transplant date or start date of BCRi therapy. Sixty-eight patients (M/F: 51/17) received an alloSCT, and 76 (M/F 53/23) were treated with BCRi (ibrutinib n=52, rituximab-idelalisib n=24). Median age was 56 (range 35-69) in alloSCT and 60 years (30-69) in BCRi (p=0.01). FISH data were available in 73% of alloSCT patients: 17pdel was positive in 66% (de novo n=22, acquired n=9, unknown n=2). FISH data were available in all BCRi patients: 17pdel was positive in 70% (de novo n=23, acquired n=22, unknown n=8). Median number of previous therapies was 2 in both groups (alloSCT: range 1-7; BCRi: range 0-8, p=0.33). Patients without del17p were refractory or early relapse in 26/68 alloSCT and 23/76 BCRi patients (p=0.37). Reduced-intensity conditioning regimen was used in 67/68 alloSCT and donor type was sibling in 29, matched unrelated in 32, haploidentical in 7 cases. Disease status before alloSCT was complete remission (CR)=21, partial remission=25, stable/progressive disease=22. The median follow-up was 36 months (1-146) and 18 (2-41) for alloSCT and BCRi group (p<0.01). Two-year PFS was 47% and 63% for alloSCT and BCRi group (p=0.18), with a cumulative incidence of relapse of 32% and 36% (p=0.53). 2-yr non-relapse mortality was 25% after alloSCT. 2-yr OS was 59% *vs* 83% in alloSCT and BCRi, respectively (p=0.004). No difference in OS was observed when alloSCT from sibling donors and CR patients were selected. These retrospective data showed that so far no significant difference in PFS of 17pdel and refractory CLL patients have been observed after either alloSCT or BCR inhibitors. The difference in OS was mainly due to the higher NRM after alloSCT.

Myeloproliferative Disorders 1

C016

SECONDARY NON HEMATOLOGICAL MALIGNANCIES IN ADULT PATIENTS WITH MASTOCYTOSIS: AN ITALIAN MULTICENTRIC SURVEY

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Mastocytosis is a clonal disease characterized by a normal life expectancy in the majority of cases. Progression from Systemic Mastocytosis (SM) to SM with an Associated Hematological Neoplasm (AHN) is an infrequent but well known event that worsen the prognosis. Conversely, there are very few data about incidence and outcome of solid malignancies in mastocytosis patients. To assess secondary primary malignancies (SPM) in mastocytosis, we evaluated 826 adult (>18 years at diagnosis) patients diagnosed and regularly followed in 6 Italian Institutions. SPM were defined as *de novo* non hematological cancers diagnosed after mastocytosis, excluding non-melanoma skin cancers due to the possible under-reporting of such neoplasms by patients themselves. Males were 450 (54%). Median age at diagnosis was 49.3 years (range 19-84). Median follow-up was 2.3 years (range 0-41). Subtype diagnoses were: Cutaneous Mastocytosis (n=46), Indolent SM (n=633), Smoldering SM (n=10), SM-AHN (n=34), Aggressive SM (n=47) and Mast cell leukemia (n=2). Fifty-four patients were classified as having mastocytosis in the skin. A total of 35 SPM were diagnosed in 34 patients (4.1%). Median age at SPM was 56.4 years (range 37-76). Median time from mastocytosis to SPM was 22 months. The overall rate of SPM was 12.8 per 1,000 person-years (95%CI: 9.1-17.6) while the rate in the general Italian population was 7.6 per 1,000 person-years (95%CI: 7.5-7.7) resulting in an increased hazard ratio of 1.7 (95%CI: 1.2-2.3). We found a clearly increased risk for melanoma (n=8, Standardized Incidence Ratio, SIR 15.9, 95%CI: 7.9-31.9) and thyroid cancer (n=3, SIR 9, 95%CI: 2.9-27.9) while a non-significant increased risk was found for prostate cancer in males (n=5, SIR 2.06, 95%CI: 0.8-4.9) and breast cancer in females (n=3, SIR 1.7, 95%CI: 0.5-5.3). All the other malignancies were sporadic (one or two cases for each cancer type) and comparison to the general population was not significant. Overall survival (OS) was significantly inferior in patients with SPM as compared to patients without SPM (5-year OS 77.6% *vs* 93.7% respectively, p=.019). Therefore, patients with mastocytosis may have a significantly higher risk of developing a secondary non hematological cancer as compared to the matched general population. Careful follow-up of these patients is warranted as malignancies may increase over time and reduce life expectancy.

C017

PROGNOSTIC FACTORS ASSOCIATED WITH A REDUCED OVERALL SURVIVAL IN PATIENTS WITH 2016 WHO DIAGNOSIS OF PREFIBROTIC AND OVERT PRIMARY MYELOFIBROSIS

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Background: The 2016 WHO classification of myeloid neoplasms established distinct criteria for prefibrotic myelofibrosis (pre-PMF) and overt, fibrotic stage PMF (overt-PMF). Recently (Guglielmelli P, Blood 2017) we reported that pre-PMF and overt-PMF are distinct diseases in terms of clinical features and outcome. *Aims:* To identify prognostic factors among pts with PMF stratified according to WHO2016 criteria. *Methods:* The study included 676 PMF from 6 Italian institutions (286 pre-PMF and 390 overt-PMF). Previously published methods were used to sequence JAK2, MPL and CALR. NGS analysis with Ion Torrent platform was used to genotype 18 genes (referred as myeloid neoplasms-associated mutations (NPAMs): all coding sequence of c-KIT, TET2, RUNX1, NRAS, KRAS, DNAMT3A, IKZF1, EZH2, TP53; hot spot of IDH1/2, SRSF2; selected exons of CBL, ASXL1, SF3B1, NFE2, SH2B3, U2AF1. Clinical and molecular data were recorded at diagnosis or within 1 yrs. The prognostic value of the molecular variables with regard to OS was estimated by the Kaplan-Meier method and Cox regression. *Results:* During the follow-up (median 4.9yrs), 323 (47.8%) pts died (32.9% in pre-PMF and 58.7% in overt-PMF) and 79 (11.7%) progressed to leukemia (8.4% and 13.6% in pre- and overt-PMF respectively). Median OS was 12.7ys (range 6.25-19.21) in pre-PMF and 5.8ys in Overt-PMF (range 5.12-6.63). Older age (>65y), leucocytosis (>25x10^9/L) anemia (<10 g/dL), thrombocytopenia (<100x10^9/L), peripheral blood blasts >1% and the presence of constitutional symptoms were significantly associated with reduced OS in pre- and overt- PMF. In pre-PMF, a worst prognosis was associated with large splenomegaly and fibrosis grade MF1 in comparison with pts without BM fibrosis. No prognostic impact was observed for higher level of LDH, mere presence of splenomegaly and pruritus.In univariate analysis the following mutational profile was associated with inferior survival in both pre- and overt-PMF: mutations in ASXL1, SRSF2, EZH2 or IDH1/2 (defining the HMR status), number of mutated genes>=2, mutations in NRAS and U2AF1. SH2B3 mutations were strong predictors of reduced OS in pre-PMF. Compared to CALR type1 mutations in both series, JAK2V617F associated with reduced OS and TN pts displayed a median OS < 3ys; in pre-PMF also CALR type2 was negative predictor. Conclusions. This study indicates that pre- and overt-PMF largely share common clinical and molecular variables as negative prognostic factors for OS.

C018

CHANGES IN MUTATION PROFILE IN MF PATIENTS DURING TREATMENT WITH JAK INHIBITORS

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Ruxolitinib (ruxo), a JAK2 inhibitor (JAKi), produced modest reduction of JAK2V617F variant allele frequency (VAF) in long-term follow-up (F-U) of patients (pts) with myelofibrosis (MF), and only a few achieved major decrease. Little is known about the fate of other mutations. Aim of the study was to evaluate changes in mutation landscape, as measured by VAF changes of driver (JAK2V617F,CALR,MPL) and 27 myeloid neoplasms-associated mutations (NPAMs), during treatment with JAKi compared to baseline (bl) in MF patients. 59 patients with WHO2016/IWG-MRT-diagnosis of PMF (=35) and PPV/PET-MF (=24) were included. Thirteen (11 PMF, 2 PPV/PET-MF) received hydroxyurea (HU) and 46 (24 MF, 22 PPV/PET-MF) a JAKi (39 ruxolitinib, 5 fedratinib, 2 fedratinib/ruxo). Mutations (ASXL1, CALR, CBL, C-KIT, CSF3R, CUX1, DNMT3A, ETNK1, EZH2, IDH1, IDH2, IKZF1, JAK2, KRAS, MPL, NFE2, NRAS, PTPN11, RUNX1, SETBP1, SH2B3, SF3B1, SRSF2, TET2, TP53, U2AF1, ZRSR2) were

analysed in granulocyte DNA by NGS, IonTorrent-PGM paltform. CALR and JAK2V617F VAF were evaluated by RTQ-PCR/CE sequencing. Mutational analysis was performed at median treatment duration of 2.7y for HU, 3y for ruxo and 1.4y for fedratinib. The presence of drivers at bl among HU- and JAKi-treated pts was similar [92.3% (8 JAK2, 4 CALR) *vs* 97.8% (38 JAK2, 6 CALR, 1 MPL)]. In 4 HU JAK2+ pts the median VAF increased by 15.6%, 1 pt +60%, while in 1 it decreased by 63%; in 1/4 CALR+ a 25% VAF reduction was observed. Among JAKi pts, in 7/38 (18.4%) JAK2VF VAF reduced by a median of 32% while 5/38 (13%) showed a median increase of 25.4%; no change was observed in CALR VAF. Among HU pts, 9/13 (69.2%) had at least 1 NPAM mutation.1 pt acquired CBL mutation (VAF 7%), in 1 the SH2B3 VAF increased from 17.6% to 46.6%, other mutations remained stable. In JAKi-treated pts: 36/46 (78.3%) presented NPAMs at bl. VAF increased (range: +29% - +399%) for 9 mutations (1 EZH2, 2 ASXL1, 3 NFE2 and 3 TET2), while it decreased in 8 (4 ASXL1, 2 SH2B3, 1 NFE2, 1 TET2). Eight pts acquired a novel mutation (VAF%) [1 EZH2 (39%), 1 SF3B1 (11%), 2 ASXL1 (6% and 7%), 2 KRAS (39% and 23%), 1 NRAS (12%), 1 PTPN11 (8%)] while in 2 pts existing mutations at bl disappeared (1 EZH2 (47%), 1 NFE2 (20%)]. The clinical relevance of fluctuations of mutations over treatment remains to be addressed in a wider series of HU-treated pts. However, these data suggest that ruxolitinib did not prevent the acquisition of novel mutations in genes associated with MF.

C019

MODELING CALR MUTATION BY GENOME EDITING IN HEMATOPOIETIC CELLS: EFFECTS ON MEGAKARYOCYTOPOIESIS

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Calreticulin (CALR) mutations are the second most frequent abnormality in myeloproliferative neoplasms (MPNs) after the JAK2V617F mutation, and their pathogenetic role is under investigation. There are several types of CALR mutations, the most frequent are a 52bp deletion (T1) and a 5bp insertion (T2) in exon9. All mutations cause a recurrent frameshift resulting in a C-terminal domain with a common novel sequence of 36 aminoacidic. We first generated mutation-specific rabbit and mouse antibodies using immunogenic peptides comprised within the common mutated domain. In order to generate cell models allowing mechanistic analysis of mutated CALR in a hematopoietic setting, we generated CALR knock-out (KO) variants starting from donors' CD34+ cells, BCR/ABL-mutated K562 and JAK2V617F-mutated HEL cells, and CALR-T1 variants from K562 and HEL cells, by using CRISPR/Cas9 editing. We also generated models of transient expression of CALR WT, T1 and T2 by vectors transfection in CALR-KO K562 cells. Characterization of modified cells and cell lines was pursued by proliferation, cell cycle and apoptotic assays. Clonogenic potential was assessed by plating CD34+ cells to form CFU-GM, BFU-E and CFU-Mk in semisolid medium. Megakaryocyte (Mk) commitment was analyzed in liquid culture of K562 CALR variants and assessed by CD41 and CD61 immunoreactivity by flow. We successfully confirmed the absence/presence of CALR variants in the various cell models and CD34+ cells by western blotting using mutation-specific antibodies. Clonogenic assay showed that CALR-KO CD34+ cells generated a number of colonies 2, 10 and 7–fold higher than WT CD34+ cells respectively for CFU-GM, CFU-Mk and BFU-E ($p<0.05$). Analysis of Mk differentiation in liquid cultures showed that K562 CALR-KO and T1 cells differentiated more efficiently than WT K562 (respectively 61% and 49% *versus* 25%, $p<0.05$). By using specific anti-mutated CALR Abs in confocal microscopy, we demonstrated the presence of mutated CALR in cytosolic compartment as well as on cells surface, arising the possibility to selectively target membrane associated CALR for diagnosis and therapeutic purposes. Overall, these data indicated that disruption of CALR alters the Mk commitment in hematopoietic cells; interestingly, some effects ascribed to CALR-T1 were mimicked by CALR KO. Therefore, cells with modeled expression of mutated CALR and specific Abs represent invaluable tools for mechanistic studies of CALR-mutated MPNs.

C020

PROGNOSTIC SIGNIFICANCE OF A COMPREHENSIVE HISTOLOGIC EVALUATION OF RETICULIN FIBROSIS, COLLAGEN DEPOSITION AND OSTEOSCLEROSIS IN PRIMARY MYELOFIBROSIS PATIENTS

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Aims: We aimed to evaluate whether a comprehensive histological evaluation of reticulin fibrosis, collagen deposition and osteosclerosis in bone marrow trephine biopsies (BMBs) of primary myelofibrosis (PMF) patients could have prognostic implication. *Methods:* According to the new grading system for the evaluation of Reticulin fibrosis, Collagen deposition and Osteosclerosis, proposed by Kvasnicka *et al.* in 2016, each of these morphological parameters was evaluated and graded from 0 to 3 in a series of 122 base-line BMBs of PMF patients. Then, a comprehensive score of the bone marrow stromal changes (RCO – score), obtained by summing the results of the three morphological parameters and thus ranging from 0 to 9 (grade of reticulin fibrosis+grade of collagen deposition+grade of osteosclerosis), was assigned to each case. *Results:* 88 out of 122 patients displayed a low-grade and 34 a high-grade RCO score. The latter was more frequently associated with anemia, thrombocytopenia, leukocytosis, peripheral blood blasts and increased lactate dehydrogenase levels. RCO score resulted strictly correlated with overall mortality ($p=0.013$) and International Prognostic Scoring System (IPSS) risk categories, and was able to discriminate the overall survival of both low- and high-grade patients (Log-Rank test: $p<0.001$). Moreover, it proved to be more accurate than the European Consensus on grading of bone marrow fibrosis (ECGMF grade) in identifying high-risk patients with poor prognosis. Finally, a combined analysis of RCO scores and IPSS risk categories in an integrated clinical-pathological evaluation was able to increase the positive predictive value (PPV) for mortality in high-risk patients. *Conclusions:* the comprehensive RCO score, obtained by histological evaluation of reticulin fibrosis, collagen deposition and osteosclerosis, resulted prognostically significant and more accurate than ECGMF grade in identifying high-risk PMF patients, and improved PPV when applied in addition to IPSS.

Non Hodgkin Lymphomas 1

C021

BRIEF CHEMOIMMUNOTHERAPY STRATEGY FOR ELDERLY PATIENTS WITH UNTREATED ADVANCED STAGE FOLLICULAR LYMPHOMA: COMPARISON OF TWO TRIALS BY FONDAZIONE ITALIANA LINFOMI

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FIL has achieved good experience in treating elderly FL patients (pts) with brief chemoimmunotherapy strategy. Aim of this retrospective analysis was to compare 2 trials specifically devised for elderly advanced stage FL requiring therapy. ML17638 trial included 241 pts (age 60-75) treated with 4 monthly R-FND courses (Rituximab, Fludarabine, Mitoxantrone, Dexamethasone) followed by 4 weekly Rituximab (R); responding pts were randomized between short R maintenance or observation; for the current purpose 101 pts randomized to observation and 32 not randomized pts (total 133) were analysed. FLE09 trial included 76 pts (age 65-80) treated with 4 monthly R-BM (Rituximab, Bendamustine, Mitoxantrone) followed by 4 weekly R infusions. Characteristics of the 209 evaluable pts were: median age 68 years (range 65-79); 118 (56%) females; 15% advanced stage II, 21% stage III and 64% stage IV. According to FLIPI pts were: 10% at low, 33% at intermediate and 57% at high risk. Two hundred and eight pts were evaluable for response: overall response was 82% with 67% complete remissions (CR), 15% partial remissions (PR), 10% stable (SD) or progressive diseases (PD) and 8% pts discontinued treatment due to adverse events (AE) without response evaluation. According to treatment scheme, responses were as follows: 80 (60%) CR, 20 (15%) PR, 17 (13%) SD/PD, 16 (12%) AE for R-FND and 59 (78%) CR, 12 (16%) PR, 5 (6%) SD/PD for R-BM. Overall, 2 and 3-years PFS were 71% and 62%, respectively. Three-years PFS was higher for R-BM regimen: 67% *vs* 59% (adjusted HR 0.59, p=0.085; Figure 1); predictive factors for worse PFS were: high FLIPI score, ECOG PS ≥1 and B symptoms; females showed a better PFS than males (HR 0.54, p=0.013).

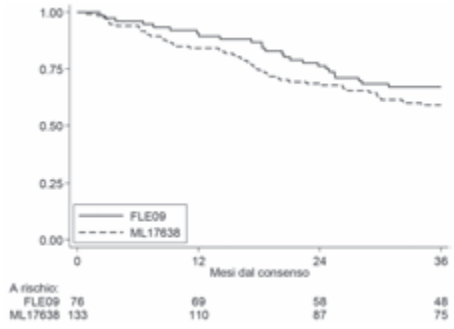

Figure 1. Progession-Free survival according to trial.

Two and 3-years OS were 92% and 86%, respectively. Three-years OS seemed to be better for R-BM *vs* R-FND without statistically significance: 92% and 83%, respectively (adjusted HR 0.56, p=0.327) with no predictive factors significantly related. The most frequent severe toxicity was neutropenia: 25% of R-FND and 18% of R-BM courses; 4 and 8 cases of neutropenic fever with 11 and 6 cases of grade 3 infections were reported respectively. In conclusions, a brief chemo-immunotherapy strategy can induce high CR rate and prolonged PFS in elderly de-novo advanced stage FL: both regimens are safe and effective; R-BM regimen seems to induce higher CR rate and better PFS, with reduced toxicity, in an older population than that one treated with Flurarabine containing regimen.

C022

DIRECT-ACTING ANTIVIRALS DURING OR AFTER IMMUNO-CHEMOTHERAPY IN HEPATITIS C VIRUS-ASSOCIATED DIFFUSE LARGE B-CELL LYMPHOMAS

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Direct-acting antivirals (DAAs) demonstrated >90% sustained virological responses (SVR) across all genotypes in hepatitis C virus (HCV)-infected patients (pts), without significant side effects. Updated international guidelines suggest HCV eradication by DAAs in pts with HCV+ diffuse large B-cell lymphoma (DLBCL) achieving complete response (CR) after 1st line immunochemotherapy (I-CT), although limited experiences substantiate this recommendation. Moreover, no data concerning concurrent administration of DAAs with I-CT have been reported. We retrospectively analyzed virological and hematological outcome and survival of 32 consecutive pts with HCV+ DLBCL treated with DAAs regimens either concurrently (Concurrent Cohort, ConC: n=7) or subsequently (Sequential Cohort, SeqC: n=25) to 1st line I-CT. Germinal-center (GC)/non-GC cases according to Hans were 37/63%. Median age was 62 years (y), IPI high/high-intermediate in 15 pts (47%). Genotype was 1 in 20 (63%), 2 in 10 (31%) 3 and 4 in 1 pt (3%). Cirrhosis was evidenced in 7 pts (22%). Seven pts (22%) previously failed interferon-based antiviral therapy (AT). I-CT was R-CHOP(-like) in 30 pts and R-ACVBP in 2. Anthracyclines dose was reduced in 10 pts. I-CT was completed in all but 3 pts. Overall, 30/31 evaluable pts obtained CR (97%), while 1 progressed. All pts received appropriate DAAs according to genotype: 30 pts sofosbuvir (SOF)-based regimens (SOF-ledipasvir in 11, SOF+ribavirin [RBV] in 10, SOF+daclatasvir in 7, SOF+simeprevir in 2) and 2 pts "3D regimen". Overall, 25/27 assessable pts achieved SVR (93%), 4/5 (80%) in ConC and 21/22 (95%) in SeqC. The 2 non-responders achieved SVR after a 2nd DAA regimen. DAAs were well tolerated, with only 7 pts (22%) experiencing 13 grade (g) 1-2 adverse events (AEs) in SeqC, while no AE was recorded in ConC.

One pt treated concurrently (14%) experienced hepatic toxicity (g 4), compared to 14 pts (56%; g 1-2 in 9, g 3-4 in 5) treated sequentially (p=0.08). At a median follow-up of 2.3 ys no pt died (OS 100%), 2 pts progressed (2y PFS 93.2%) and 1 developed hepatocellular carcinoma (2y EFS 88.5%) (Figure1). IPI, ≥2 extranodal sites and albumin <3.5 g/dl retain prognostic value on PFS (p<0.01). Excellent outcome of this selected retrospective series suggests benefit of HCV eradication by DAAs either after or during I-CT in HCV+ DLBCL. Moreover concurrent DAAs and R-CHOP administration resulted feasible and effective and may prevent hepatic toxicity of I-CT.

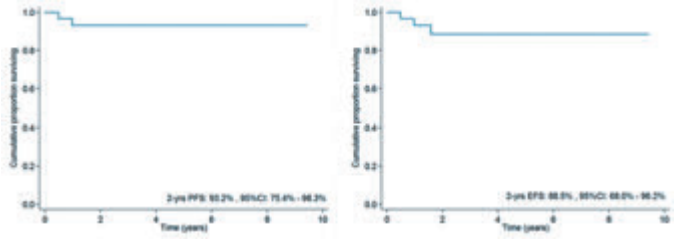

Figure 1.

C023

OBINUTUZUMAB-MINICHOP FOR THE TREATMENT OF ELDERLY UNFIT PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA. A MULTICENTRE PHASE 2 STUDY BY THE FONDAZIONE ITALIANA LINFOMI

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We conducted a prospective phase 2 study to evaluate activity and safety of Obinutuzumab (GA101)-miniCHOP combination in elderly unfit patients (pts) with DLBCL. Elderly subjects (≥65 years) with a newly diagnosed DLBCL were considered eligible if resulted unfit at Comprehensive Geriatric Assessment. Patients received miniCHOP (400 mg/m^2 cyclophosphamide, 25 mg/m^2 doxorubicin, 1 mg vincristine on day 1 of each cycle, and 40 mg/m^2 prednisone on days 1-5) every 21 days, combined with GA101 1000 mg on day 1,8,15 of cycle 1 and on day 1 of subsequent cycles. The use of G-CSF was mandatory. The primary endpoint was Complete Response Rate (CRR) according to Cheson 1999 criteria. Secondary endpoint was safety. The sample size was estimated according to an optimal Simon two-stages design. The study was designed to assess whether GA101-miniCHOP could increase the CRR compared to historical data. The null hypothesis (p0) has been set equal 0.60 on the basis of what reported by Peyrade et al (Lancet Oncol, 2011) and the alternative hyp. (p1) was set at 0.75, with a type I and II error of 10% and 90%. Stage 2 could be opened with at least 22 CR out of the first 34 pts. Analysis was by intention to treat. We here report the results of the stage 1. From August 2015 to June 2016 were enrolled 34 pts and 1 patient was subsequently excluded. Median age was 82 yrs (68-89), 18 were males and IPI was 3-5 in 21 cases. Overall 228 cycles were delivered and 27 pts completed all 6 planned courses. Four pts interrupted treatment because of adverse events (AE), and 2 because of lack of response. Final response was reported as CR in 14 pts (42%), and partial in 8 (24%); 10 pts had stable or progressive disease (30%) and 1 patient (3%) was not assessed. Pts who experienced AEs were 28: hematological grade 3-4 AEs included neutropenia (13 cases; 39%), and thrombocytopenia (1 case, 3%); grade 3-4 non hematological AEs occurring in more than one case included skeletal muscle (2 cases, 6%) and metabolic disorders (3 cases, 9%). With the observed CR rate, enrollment was interrupted according to what planned for the interim analysis. Even if the study couldn't be completed after interim analysis, GA101-miniCHOP is active and well tolerated for the treatment of elderly unfit pts affected by DLBCL. With these results we were not able to demonstrate the initial study hypothesis that GA101-miniCHOP could improve results of historical data obtained with R-miniCHOP in this setting of patients.

C024

MYC NUMERICAL ABERRATIONS UNFAVORABLY IMPACT ON DISEASE OUTCOME IN PATIENTS WITH DIFFUSE LARGE B-CELL AND HIGH-GRADE LYMPHOMA

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Introduction: Approximately 10% of DLBCL are characterized by MYC translocation, which negatively impacts on patient's (pt) survival. Also MYC numerical aberrations may influence prognosis, but their incidence and correct identification is controversial. In the present study, we analyzed the clinical outcome of pts with MYC increased copy number (ICN) in the setting of aggressive B-cell lymphoma. *Methods:* From 2011 to 2016 at our Institution, 408 consecutive pts were diagnosed with DLBCL: 354 *de novo* and 34 transformed (TL) DLBCL, and 20 B-cell lymphoma, unclassifiable (BCLU). A FISH study using split signal DNA probes specific for MYC, BCL2, BCL6 was done, scoring 60 evaluable nuclei with complete signals. MYC ICN was defined as >3 copies identified by FISH. A "cloud-like" FISH pattern due to countless copies of MYC was defined as "amplification". MYC expression by immunohistochemistry (IHC) was positive if >40%. *Results:* MYC translocation (MYC-T) was found in 33 (8%) of pts, ICN (MYC-ICN) in 56 (13.7%), and amplification (MYC-AMP) in 4 (1%) (Figure 1D). MYC-T included 9 single-hit (SH), 18 double-hit (DH) and 6 triple-hit (TH) DLBCL, with similar overall OS (p 0.9). BCLU clustered in the MYC-T group (p 0.03), whereas >80% of MYC-ICN pts had DLBCL (p 0.06). MYC IHC was strongly positive in >90% of pts with MYC-T and MYC-ICN>4. MYC-ICN included 3-10 copies/cell in 47 pts (9 not evaluable). Considering overall survival (OS) of pts with different numbers of MYC extra-copies, pts with ≤4 copies had a better OS compared to >4 (p=0.04, Figure 1A).

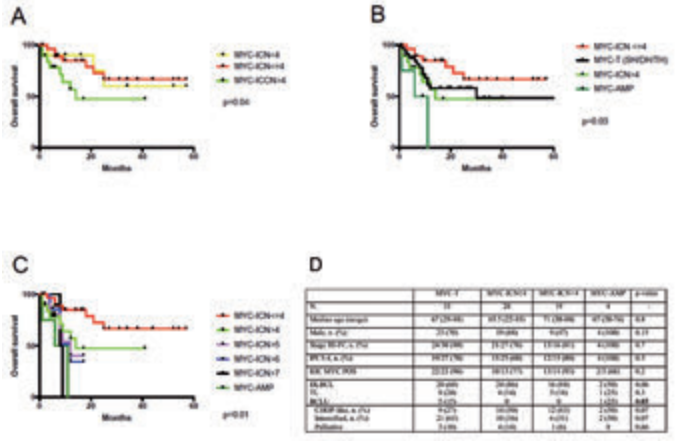

Figure 1.

Compared to MYC-T, OS of MYC-ICN >4 was similar and OS of MYC-ICN ≤4 was better (p=0.03, Figure 1B). Moreover, there was a negative correlation between increasing numbers of extra-copies and survival, with MYC-ICN>7 and MYC-AMP having the worst prognosis (p=0.01, Figure 1C). As such, according to MYC-ICN pts could be di-

vided into 4 groups (Figure 1D). Intensified therapy (R-DA-EPOCH, Burkitt's-like or ASCT consolidation) was given to 54% of pts, with no significant advantage. *Conclusions:* Our study shows that MYC-ICN is frequent in aggressive B cell lymphomas, and provides evidence that a copy number ≤4 or >4 is a cut-off of prognostic relevance. We demonstrated that the number of gene copies directly correlates with worsening prognosis, MYC-ICN>7 and MYC "amplification" being associated with the poorest outcome. Further investigation is needed to clarify the biological implications and the applicability of specific therapeutic strategies.

C025

RITUXIMAB AND DOSE ADJUSTED EPOCH FOR THE TREATMENT OF DOUBLE-/OR TRIPLE HIT AND DOUBLE EXPRESSOR DIFFUSE LARGE B-CELL LYMPHOMAS AT DIAGNOSIS

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Introduction: Diffuse large B-Cell Lymphomas (DLBCL) with chromosomal rearrangements involving MYC, BCL2 and/or BCL6 (DHL/THL) or with double expression of MYC and BCL2 proteins (DE) have an inferior outcome when treated with R-CHOP chemotherapy. The optimal induction therapy for these lymphomas has not yet defined. Preliminary data have suggested superior complete responses following Dose Adjusted EPOCH plus Rituximab (RDAEPOCH). *Methods:* Between 2013 and 2017, we conducted a prospective observational study to evaluate the outcome of DHL/THL and DE lymphomas after 6 courses of RDAEPOCH. In all patients (pts) central nervous system (CNS) prophylaxis was planned. Eligibility criteria were biopsy proven diagnosis of DLBCL DHL/THL (identified by recognition of rearrangements of MYC, BCL2 and/or BCL6 obtained by fluorescent *in situ* hybridization) or DE [determined by immunohistochemical analysis (MYC expression ≥40% and BCL2 ≥50% of tumor cells)], age between 18 and 78 years and adequate organ functions. Cell of origin (COO) was defined according to Hans algorithm (nanostring evaluation of COO is on going). *Results:* Thirty-seven pts were included, 8 were DHL/THL (n=4 MYC/BCL2, n=2 MYC/BCL6, n=2 THL) and 29 DE. According to Hans algorithm, 20 (n=8 DHL/THL, n=12 DE) and 17 (all DE) pts had a germinal center B-cell subtype (GCB) and non GCB, respectively. The median age was 60 years (range, 29-78 years). Eighteen (49%) and twenty (54%) had high-intermediate/high risk score as defined by International Prognostic Index (IPI) and enhanced International Prognostic Index (NCCN IPI), respectively. Pts received a median of 6 courses (range, 1-6). After treatment, 23 (62%) achieved a CR, 7 a PR (19%) and 7 had PD (one CNS relapse). Early response was associated to a better outcome [no relapse between pts achieving CR after 3 cycles and PFS of 60% for those in PR]. The median time to failure was 163 days (49-275 days). Four out of 37 pts did not conclude the treatment for PD (n=2), consolidation with autoSCT (n=1), and infectious complications (n=1). At a median follow-up of 300 days (range, 90-1054), the estimated 1-year PFS and OS for the entire population were 70% (95%CI, 50%-83%) and 95% (95%CI, 71%-99%). The estimated 1 year PFS was 76% (95%CI, 54%-88%) *versus* 46% (95%CI, 8%-88%) in DE (median PFS not reached) and DHL/THL (median PFS 275 days), respectively. (p=ns). *Conclusions:* RDAEPOCH is feasible with promising efficacy, especially in DE lymphomas.

Hemostasis and Thrombosis

C026

EFFECTIVENESS AND SAFETY OF RFVIIA IN PAEDIATRIC GLANZMANN THROMBASTHENIA PATIENTS: DATA FROM THE INTERNATIONAL GLANZMANN THROMBASTHENIA REGISTRY

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Introduction: Platelet transfusion is the standard treatment for Glanzmann thrombasthenia (GT); however, recombinant activated factor VII (rFVIIa; NovoSeven®) has been shown to be effective in GT patients with platelet antibodies and past or present refractoriness to platelet transfusions. We report data from the Glanzmann Thrombasthenia Registry (GTR) on the effectiveness and safety of rFVIIa when used to treat and prevent surgical and non-surgical bleeds in children with or without platelet antibodies and/or refractoriness. *Methods:* Data on bleed treatment and outcomes for children aged <18 years with congenital GT were prospectively collected in the GTR, which is an international, multicentre, observational, post-marketing study of rFVIIa. Effectiveness analyses were based on all patients and treatment-allocated bleeds (n=634) for which efficacy outcomes were known; all patients and bleeds (n=643) were included in safety analyses. *Results:* Between 2007 and 2011, 27 children were treated for 44 surgical procedures (minor 36, major 8); 104 children were treated for 599 non-surgical bleeds (severe 145, moderate 454; spontaneous 423, post-traumatic 176). Half of all minor procedures (18/36; 50.0%) were treated with rFVIIa, either alone or in combination with antifibrinolytics (AF) or platelets (P)±AF (other 50.0% received P±AF or AF), while major procedures were treated most frequently with rFVIIa+P±AF (3/8; 37.5%). Of 590 non-surgical bleeds evaluated for effectiveness, 205 (34.7%) were treated with rFVIIa alone or in combination with AF or P±AF (other 65.3% received P±AF or AF). Overall, the effectiveness of treatment for minor procedures/major procedures/non-surgical bleeds was 100.0%/100.0%/89.3% for rFVIIa alone, 100.0%/100.0%/84.2% for rFVIIa+AF, 91.7%/100.0%/75.7% for P±AF, and 83.3%/0.0%/73.3% for rFVIIa+P±AF. Of 25 adverse events (AEs) reported overall from the 643 admissions included in the safety analysis, nine occurred in rFVIIa-treated patients, but all were considered unlikely to be related to rFVIIa. There were no thromboembolic events. *Discussion and Conclusions:* Regardless of platelet antibody or refractoriness status, rFVIIa (administered with or without P±AF) provided effective haemostasis with a low frequency of AEs when used for surgery and to treat non-surgical bleeds in paediatric GT patients.

C027

NONACOG BETA PEGOL IN ADULT AND PAEDIATRIC PATIENTS: POOLED DATA FROM THE PARADIGM™ CLINICAL PROGRAMME

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Objectives: Nonacog beta pegol (N9-GP) is an extended half-life recombinant glycoPEGylated factor IX (FIX). We present pooled N9-GP data from 5 completed trials conducted in previously treated paediatric, adolescent and adult haemophilia B patients (PTPs) (1 phase 1; 4 phase 3 trials, including the pivotal randomised trial). *Methods:* Results from patients who received N9-GP 40 IU/kg (all ages) or 10 IU/kg (adolescent/adult only) once-weekly prophylaxis including treatment of bleeds are summarised. This analysis includes pharmacokinetics, safety and haemostatic efficacy. *Results:* 115 male PTPs (FIX <=2%) were included: 72 adults (18–65 years), 18 adolescents (13–17 years) and 25 children (0–12 years), with a total of 8801 exposure days to N9-GP. In the phase 3 trials, 30 patients received N9-GP at 10 IU/kg/week, 54 patients received 40 IU/kg/week and 15 were treated on-demand. No inhibitors or thromboembolic events were observed. Of 54 (47%) patients treated weekly with 40 IU/kg, 23 (43%) experienced zero bleeding episodes. Median overall annualised bleeding rate (ABR) for all age groups on 40 IU/kg was 1.03 (IQR 0.00–2.89) and median spontaneous ABR was 0.00 (IQR 0.00–0.80). ABR was lower in adolescents/adults randomised to 40 IU/kg than 10 IU/kg (p<0.05). Overall success rate for the treatment of bleeds was 93%; most bleeds (87%) resolved after a single injection. Adults, adolescents and children showed single-dose (40 IU/kg) half-lives of 83, 89 and 73 hours, respectively, and incremental recoveries of 0.023, 0.020 and 0.016 (IU/mL)/(IU/kg), respectively. Estimated mean steady-state FIX trough levels with weekly 40 IU/kg were >=0.15 IU/mL in all age groups. 13 adolescent/adult patients receiving 40 IU/kg N9-GP once-weekly prophylaxis had collectively 20 target joints at study start; by the end of the extension trial, all target joints had resolved. Two questionnaires (EQ-5D VAS and Haem-A-QoL) in adults/adolescents demonstrated significant improvements in quality of life (QoL) from baseline to end of trial in those receiving 40 IU/kg; by the end of the trial, patient QoL scores approached that of the general population. *Conclusions:* N9-GP was well tolerated and effective in preventing bleeding at 40 IU/kg once weekly, maintaining FIX activity levels >=15% across all age groups. Once weekly prophylaxis resolved existing target joints and improved patient QoL in adults/adolescents.

C028

THALIDOMIDE FOR HEREDITARY HEMORRHAGIC TELANGIECTASIA: EFFICACY AND SAFETY OF LONG-TERM TREATMENT

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Hereditary hemorrhagic telangiectasia (HHT) is a genetic disease that leads to multiregional angiodysplasia. Severe recurrent epistaxis is the most common presentation often leading to severe anemia. In a previous phase 2, non-randomized, single-centre study, we have shown that low-dose thalidomide (thal) was safe and effective in reducing HHT epistaxis, providing a rapid, long-lasting clinical improvement (Lancet Haematol 2015; e465-73). However, after the end of therapy, most patients relapsed at various time points. The present study is an extension of our previous clinical trial (ClinicalTrials.gov Identifier: NCT01485224) to assess the effects of thal on the severity of epistaxis in HHT subjects who relapse after a first successful treatment with the drug, and to evaluate safety and tolerability. HHT patients, successfully treated with thal, may receive again thal providing that they relapsed at least 4 weeks after the end of previous therapy. Thal is given orally for 8 weeks at the same dosage that induced remission (50 or 100 mg/day). Thal courses may be repeated at most 3 times; in case of relapse occurring within 4 weeks from the end of a previous course, thal is permanently discontinued. Monthly follow-up is based on the epistaxis severity score and transfusion need, with adverse events being reported. Fourteen HHT patients, 9 M and 5 F, aged 48-84 years (median 64), with mutations in either ACVRL1 (12 cases) or ENG gene (2 cases), who relapsed at 10-68 weeks (median 32) after the end of thal induction therapy, have been retreated, 9 patients with thal 50 mg/day and 5 with 100 mg/day. Thal courses were effective in 13 (93%) cases with a significant reduction of nose bleeding, increase of Hb levels and decrease of the transfusion need. At a median follow-up of 81 weeks, range 39-117, after the end of the first retreatment course, 3 (22%) cases maintained a response, whereas 10 (71%) relapsed again with a median relapse-free survival of 30 weeks. Nine patients were successfully retreated with a second course and 5 patients, who relapsed again, with an additional third course. Median relapse-free survival was 28 weeks after the end of the second course and 24 weeks after the third course. Only nonserious adverse effects were observed, including constipation and drowsiness. These results strongly support the hypothesis that repeated administrations of thal maintain their efficacy and can be used for long-term treatment of HHT epistaxis.

C029

RAPID DIAGNOSTIC APPROACH WITH AUTOMATIC TESTS IN A COHORT OF CHINESE PATIENTS WITH VON WILLEBRAND DISEASE: RESULTS FROM THE CHINESE-ITALIAN CREWILACT STUDY

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Background: von Willebrand disease (VWD) is the most common inherited bleeding disorder and is due to quantitative and/or qualitative defects of von Willebrand factor (VWF). Despite its improved knowledge, only a few data on the correct diagnosis of VWD requiring treatments have been available so far in both developed and developing countries. *Aims:* a cross sectional study with automatic VWF tests, [Chinese Registry of von Willebrand disease with Instrumentation Laboratory approach using Automatic Tests (CReWILAcT)] was designed by the Centers of Suzhou and of Milan to evaluate to most rapid and simple approach to VWD diagnosis. *Methods:* Chinese and Italian healthy individuals were used as normal controls while VWD patients were diagnosed in Suzhou according to the ISTH-SSC-SC-VWF criteria. The 3 main VWF activities (VWF:RCo, VWF:CB, VWF:Ag) were measured in citrated plasma by the automatic ACL-ACUSTAR systems using commercially available reagent kits. The data of these 3 tests were expressed *versus* the International Standard in IU/dL and their sensitivity and reproducibility (CV) calculated. The VWF:RCo/Ag and VWF:CB/Ag ratios were also calculated to distinguish VWD1 from VWD2A, VWD2B or VWD2M (JTH 2005; 3:2689). 3 steps were planned: a) blind exercise performed in lyophilized samples from 2 normal individuals and 6 cases with known VWD types available at NIBSC of London, UK (JTH 2011; 9:220); b) in 70 Chinese and 65 Italian healthy subjects with O and non-O blood groups; c) in 108 VWD followed in China. *Results:* Sensitivity (CV) of VWF:RCo, VWF:CB, VWF:Ag were <1 (8%), <1 (7%) and <1 (6%), respectively. In step a, in two different exercises, both labs could confirm diagnosis of normal controls or VWD2A, VWD2B and VWD2M. In step b, VWF levels (Mean+SD) of O blood groups were lower than non-O in both Ch (34/36) and It (32/33) cases: VWF:Ag [Ch=89+51/102+38); It=70+26/102+38); VWF:RCo [Ch=97+36/114+33); It=74+29/101+31); VWF:CB [Ch=87+27/102+25); It=77+25/114+33). In step c, in all VWD, the total mean+SD (range) of activities were: VWF:Ag [16+14(<1-67)]; VWF:RCo [13+12(<1-46); VWF:CB [13+13 (<1-47)]. Using VWF:RCo/Ag VWF:CB/Ag ratios VWD3(24), VWD1/2N(43), VWD2A(21), VWD2B(8), VWD2M(13) could be confirmed. *Conclusions:* These results support the use of such an automatic VWD diagnosis in routine labs world-wide.

C030

MULTISTEP TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA ASSOCIATED WITH ISOLATED THROMBOCYTOPENIA: A CASE REPORT

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Background: Treatment of symptomatic Paroxysmal Nocturnal Hemoglobinuria (PNH) relays on eculizumab, a monoclonal antibody which antagonizes the C5 component of complement, resulting in dra-

matic decrease of hemolysis. PNH is often associated with venous thrombotic events (VTE) which require long-term anticoagulation with vitamin k antagonists (VKA). *Aims:* to describe a patient who was successfully managed with triple therapy including eculizumab, VKA and low dose eltrombopag (Elt) to correct the concomitant thrombocytopenia. Case report. A 35 yr old female patient (pt), with a history of autoimmune chronic tyroiditis, was referred to our Center for worsening of chronic thrombocytopenia. Routine screening for suspected immune-mediated thrombocytopenia (ITP) revealed mild splenomegaly and portal vein thrombosis. Screening for congenital thrombophilia was negative; cytofluorimetry was performed: 6 colours method; markers: CD45, CD59, CD235a, CD33, CD15, CD14, CD24, FLAER. The test was consistent with presence of a PNH clone on 85% of neutrophils, 70% of monocytes and 4% of erythrocytes. Shortly after diagnosis of PNH, she was admitted to the hospital for abdominal pain and a CT scan revealed thrombotic occlusion of sovra-hepatic and splenic veins. Therefore, eculizumab was started, along with VKA treatment. Because of persistently low plt counts (25-35 x 10*9/L) she underwent bone marrow biopsy (cellularity 30%; megakariocytic hyperplasia) and plt survival study (plt life span of 6 days with splenic uptake) which were consistent with ITP associated with PNH and Elt was started. Figure 1 shows response to treatment (eculizumab) with rapid lowering of LDH levels and increased plt counts after eltrombopag therapy start. The pt is currently on triple therapy with eculizumab, VKA and eltrombopag 75 mg/day; no additional thrombotic complications have occurred. *Comments:* The case described is peculiar in that two rare disorders occurred in the same pt: coexistence of ITP made treatment VKA of PNH-related thrombosis challenging: a triple therapy regimen was therefore felt to be appropriate. Elt was preferred for its recognized effectiveness in ITP and its safety profile in BM aplasia. In this pt, the reported potentially increased risk of thrombotic events related to Elt use was counterbalanced by the concomitant VKA therapy and no additional thrombotic events developed.

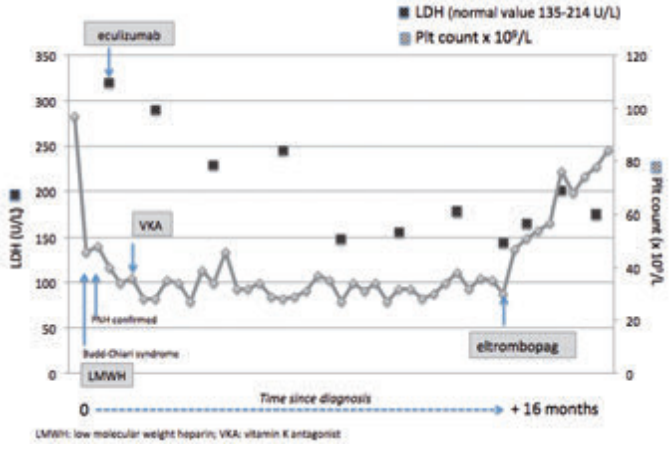

Figure 1. LDH and plt count before and during combined treatment with eculizumab and eltrombopag.

Chronic Myeloid Leukemia

C031

BOSUTINIB (BOS) VS IMATINIB (IM) FOR NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA (CML): INITIAL RESULTS FROM THE BFORE TRIAL

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University of Milano-Bicocca, Italy, University of Utah, USA, Memorial Sloan Kettering Cancer Center, USA, Singapore General Hospital, Duke-NUS Graduate Medical School, Seoul St. Mary's Hospital, Avillion LLP, Avillion LLP, Pfizer Inc, Pfizer Global Research and Development, Klinik für Innere Medizin II, Universitätsklinikum Jena, Universitätsklinikum RWTH Aachen, University of Texas MD Anderson Cancer Center, USA

Background: BOS is a potent SRC/ABL tyrosine kinase inhibitor approved for treatment of adults with Ph+ CML resistant/intolerant to prior therapy. We assessed the efficacy and safety of BOS *vs* IM as first-line treatment of chronic phase (CP) CML. *Methods:* In this ongoing, multinational, phase 3, open-label study (NCT02130557), 536 patients (pts) with newly diagnosed CP CML were randomized 1:1 to BOS 400 mg QD (n=268) or IM 400 mg QD (n=268 [3 not treated]). Per protocol, efficacy was assessed in a modified intent-to-treat (mITT) population of 487 Ph+ pts (BOS, n=246; IM, n=241) with e13a2/e14a2 transcripts; Ph– pts and those with unknown Ph status and/or atypical BCR-ABL transcript type were excluded from this population. *Results:* After ≥12 mo of follow-up, 78.0% of BOS and 73.2% of IM pts remain on treatment with median durations of 14.1 and 13.8 mo, respectively. Major molecular response (MMR) rate at 12 mo (primary endpoint) was significantly higher with BOS *vs* IM in the mITT population (47.2% *vs* 36.9%; P=0.02) as well as in the ITT population of all randomized pts (46.6% *vs* 36.2%; P<0.02). Rate of complete cytogenetic response (CCyR) by 12 mo was also significantly higher with BOS (77.2% *vs* 66.4%; P<0.008) in the mITT population and cumulative incidence of MMR (hazard ratio, 1.34; P<0.02) and CCyR (hazard ratio=1.38; P≤0.001) was favorable with BOS. Rates of BCR-ABL transcripts ≤10% (Intl Scale) at 3 mo (75.2% *vs* 57.3%), MR^4 at 12 mo (20.7% *vs* 12%) and $MR^{4.5}$ at 12 mo (8.1% *vs* 3.3%) were higher with BOS *vs* IM (all P<0.025). 1 BOS pt and 4 IM pts discontinued treatment due to progression to accelerated or blast phase. There were no deaths within 28 d of last dose of BOS and 4 with IM. 12.7% of BOS and 8.7% of IM pts discontinued due to drug-related toxicity. Gr ≥3 diarrhea (7.8% *vs* 0.8%) and increased alanine (19% *vs* 1.5%) and aspartate (9.7% *vs* 1.9%) aminotransferase levels were more common with BOS. Cardio-, peripheral- and cerebrovascular events were infrequent (all grades: 3%, 1.5% and 0% BOS *vs* 0.4%, 1.1% and 0.4% IM; gr ≥3: 1.5%, 0% and 0% *vs* 0%, 0% and 0.4%). *Conclusions:* Pts on BOS had significantly higher rates of 12-mo MMR and CCyR and achieved responses faster than those on IM. Consistent with the known safety profile, higher incidences of gastrointestinal events and transaminase elevations were observed with BOS. Primary results from this study suggest BOS may be an important treatment option for pts with newly diagnosed CP CML.

C032

COMPARATIVE MONITORING OF MINIMAL RESIDUAL DISEASE BY QPCR AND DIGITAL-PCR IN CHRONIC MYELOID LEUKEMIA PATIENTS ACHIEVING MAJOR OR DEEP MOLECULAR RESPONSE WITH TIROSIN-KINASE INHIBITORS

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Quantification of BCR-ABL1 transcript by qPCR is mandatory to monitor the response to TKIs therapy in CML patients. The achievement of Major or Deep Molecular Response (MMR or DMR) with TKIs is crucial for long-term survival and for treatment free remission (TFR). Currently, 60% of deep responders who discontinue the treatment loose DMR and re-challenge conituous therapy. qPCR has some intrinsic limits and it does not appear an optimal tool to select the best candidates to TKIs discontinuation. Using qPCR and dPCR (QS3D Digital PCR System), we comparatively analyzed 228 peripheral blood samples from 57 CML patients with MMR (14) or DMR (43) in at least 3 time points. Absolute quantification of BCR-ABL1 transcript by dPCR were expressed as number of BCR-ABL1 copies/ul of reaction. Patients were divided into 3 groups corresponding to the MR classes at the first time point: MR3.0, MR4.0 and MR4.5-5.0. dPCR Positive Predictive Value (PPV) was also preliminary evaluated in 14 patients undergoing TKI discontinuation. Analyzing the time course of MR in the patients of the 3 groups it was observed a similar trend, while dPCR allowed to appreciate that, at the time of starting the monitoring, patients showed different levels of BCR-ABL1 copies/ul. Furthermore, those patients with MR4.5-5.0 undetectable by qPCR resulted with detectable BCR-ABL1 transcript levels by dPCR. Secondly, while MRD measured by qPCR appear to be more homogeneous, the quantitations measured by dPCR seem more heterogeneous because of the high sensitivity and accuracy of dPCR. Therefore, dPCR values, reflecting the heterogeneity of MRD level in patients belonging to the same MR group, suggest a higher accuracy in patients stratification (Figure 1a). dPCR value of 0,468 copies/ul, previously reported as value discriminating between major responders and deep responders, was used as threshold for dPCR data analysis. Patients with absolute value of BCR-ABL1 lower than 0,468 copies/ul at the first time point presented more stable disease levels than the patients with absolute value of BCR-ABL1 higher than 0,468 copies/ul (Figure 1b). In 14 CML patients who discontinued TKIs, a preliminary analysis showed that 80% of patient with BCR-ABL1<0,468 copies/ul at discontinuation, maintained stable TFR (PPV of 80%). This study suggests that dPCR is more precise and sensitive than qPCR when detecting levels of BCR-ABL1 transcript and that dPCR seems to be more robust and accurate for CML patients stratification.

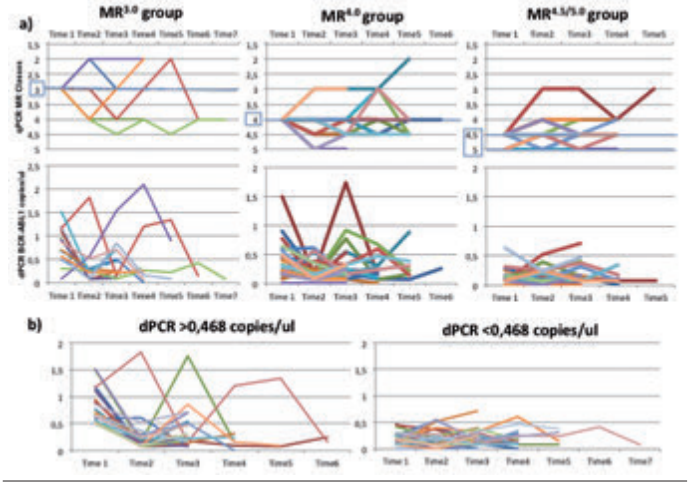

Figure 1.

C033

DETECTION OF CD26+ LEUKEMIC STEM CELLS IN PERIPHERAL BLOOD BY FLOW CYTOMETRY: A SIMPLE AND RAPID NEW DIAGNOSTIC TOOL FOR CHRONIC MYELOID LEUKEMIA

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Diagnosis of Chronic Myeloid Leukemia (CML) implies documenting in bone marrow (BM) or in peripheral blood (PB) Philadelphia (Ph) chromosome by cytogenetics and BCR-ABL1 fusion by FISH or RT-PCR. Lately, a specific co-expression of dipeptidylpeptidaseIV (CD26) within the CD34+/CD38 /Lin stem cell fraction appeared a robust biomarker for identifying CML LSCs in BM. We recently demonstrated that CD34+/CD38-/CD26+ LSCs can be easily identified by flow-cytometry also in PB during TKI therapy. We here investigated accuracy and specificity of CD34+/CD38-/CD26+ assessment in PB as a new diagnostic tool in 134 pts with clinical suspicion of CML. All pts were evaluated for PB CD26+LSCs, cytogenetics, FISH and/or BCR-ABL1 RT-PCR analysis; in 62/134 pts CD26+LSCs were tested also in BM. We used a flow-cytometry 4-color staining procedure. $2.0x10^6$ leucocytes were incubated with CD45V500 (c.2D1), CD34FITC (c.581), CD38APC (c.HIT2), CD26 (c.M-A261) and negative controls (BD Pharmigen). Acquisition and analysis of at least $1.0x10^6$ CD45+ cells were done by FACSCanto II with DIVA8 software (BD, Biosciences). In 104/134 pts we showed CD34+/CD38-/CD26+ LSCs in PB and in all of them CML was confirmed by cytogenetics, FISH and RT-PCR analysis. Median value of circulating PB CD26/ L was 15,49 (range 0,12-698) and a positive correlation with leukocyte count ($p<0.01$) was found. All CD26+ PB-BM matched pairs (57/62) showed superimposable results in terms of absolute number of CD26+LSCs/ L (18,28 and 18,38 respectively) while the percentage of CD26+ cells within the CD34+/CD38- fraction appeared lower in BM than in PB samples (median 28,18 and 36,86; range 0,55-77,14 and 5,59-98,57 respectively). In 30/134 (22.3%) PB samples and in 5/62 BM samples CD26+ LSCs were not detected and no one was found Ph or BCR-ABL1 positive. Pts with CD26 neg PB/BM samples were subsequently diagnosed as Idiopathic Myelofibrosis, Myelodysplastic/Myeloproliferative disorders, benign neutrophilia and Ph+ acute lymphoblastic leukemia. Flow-cytometry evaluation of PB CD34+/CD38-/CD26+ LSCs is a feasible, very rapid and highly specific alternative/complementary diagnostic tool for CML. To validate these data in a larger cohort of patients we are developing a pre-titrated lyophilized antibody mixture (lyotube, BD Biosciences) to maximize sensitivity and to optimize standardization and working time, with the further aim to monitor stem cells minimal residual disease in CML patients.

C034

THE IMPACT OF DIFFERENT BCR-ABL1 TRANSCRIPT ON OUTCOME OF CHRONIC MYELOID LEUKEMIA (CML) PATIENTS: A MONOCENTRIC REAL LIFE EXPERIENCE

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Background: The prognostic significance of the bcr-abl1 transcripts has been reported from patients (pts) treated with interferon (Shepherd,1995) or tyrosine kinase inhibitors (TKI) (Lucas,2009). Improved response has been reported in pts carrying the b3a2 transcript compared with those with the b2a2 transcripts after TKI treatment (Hanfstein,2014; Jain,2016). *Aims:* To asses the characteristic of pts with different bcr-abl1 transcript and its relevance in a long term follow up (FU) to the maintenance of a sustained deep molecular response (DMR) (≥MR4 according to ELN criteria), the prerequisite to TKI discontinuation. Frequencies were compared by Fisher's exact test. *Results:* 170 pts with chronic phase CML were consecutively treated at Hematology of Spedali Civili in Brescia from 2005 to 2016. Pts characteristics are described in table 1. 102 pts expressed b3a2 (60%) while 66 b2a2 (39%). Two pts without bcr-abl1 transcript characterization were excluded from analysis. After a median FU of 83 months, 93 pts (55.4%) are still DMR. The two subgroups with different transcript did not differ in Sokal score, sex, median age, frontline TKI treatment or in the number of line of treatment (1 *vs* ≥1). In contrast, they significantly differed in achievement of DMR. Pts with b3a2 transcript had a higher DMR rate compared to b2a2 (65.7% *vs* 40.9%,p 0.0016). First-line TKI impacted on DMR achievement (49% imatinib *vs* 69% 2ndGTKI;p 0.02). This difference was maintained considering the two type of transcripts being

Transplantation 1

C036

INTERMEDIATE-DOSE VERSUS LOW-DOSE CYCLOPHOSPHAMIDE FOR AUTOLOGOUS PERIPHERAL BLOOD STEM CELL MOBILIZATION IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Cyclophosphamide (CY) plus G-CSF is conventionally used to mobilize peripheral blood stem cells (PBSC) in multiple myeloma (MM) patients (pts) eligible for autologous stem cell transplantation (ASCT). However, the optimal dose of CY after novel induction regimens has not yet been clearly defined. To address this issue, we evaluated the impact of two different doses of CY on PBSC collection, in the context of the prospective, multicenter, phase III EMN02/HO95 MM trial aimed at comparing bortezomib-melphalan-prednisone *vs* high dose melphalan and ASCT as intensification therapy for newly diagnosed MM pts aged ≤65 years. According to the initial study design, the intensification phase was preceded by 3 cycles of bortezomib-cyclophosphamide-dexamethasone (VCD), followed by an intermediate dose (ID) of CY at 4 g/m^2 plus G-CSF (10mcg/Kg/day) to mobilize PBSCs. The study design was subsequently amended to increase the number of VCD cycles to 4, and to reduce the dose of CY to 2g/m^2. We analyzed 635 pts enrolled in Italian centers who received either ID-CY (n=415) or low-dose cyclophosphamide (LD-CY) (n=220). The main characteristics at baseline were well balanced between the two groups. The planned yield of 4 106 CD34+ cells/Kg was achieved in 96.6% of pts in the ID-CY group and in 94.5% of those in the LD-CY group (p=0.294). The median number of collected CD34+ cells was 10.4 106/Kg in both groups and was achieved after a median of 1 *vs* 2 leukapheresis, respectively (p=0.006). The median time to PBSC collection was 11 days in both groups (IQR: 10-12 days after ID-CY and 10-11 after LD-CY). Hematologic toxicity was more severe with ID-CY compared to LD-CY, as reflected by a higher requirement to receive red blood cell or platelet support (12% *versus* 7%, p=0.05). An univariate analysis of patients-, disease-, and response-related variables potentially predictive for successful PBSC mobilization revealed a significant relationship between baseline Plts> 150.000/mmc (p=0.001) and absence of high-risk cytogenetic abnormalities (p=0.009), and a CD34+ cell harvest ≥4 10^6/Kg. PLT > 150.000/mmc retained an independent prognostic value in a multivariate Cox regression analysis (OR= 2.95; CI 0.45-2.43; p=0.015). With a median follow-up of 28 (IQR 17-45) months, PFS estimates in ID-CY and LD-CY groups were 74% and 71%, respectively (p=0.67). In conclusion, the LD-CY is at least equally successful as ID-CY for PBSC mobilization with reduced hematological toxicity.

C037

LONG TERM FOLLOW UP IN HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR THALASSEMIA: A LARGE SINGLE-CENTER COHORT OF STUDY OVER 30 YEARS

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Centro Trapianti Midollo Osseo, Ospedali Binaghi & Microcitemico, CIC[881]

Although hematopoietic stem cell transplantation (HSCT) represents the only curative treatment for patients with thalassemia major, only a few studies have focused on the long-term follow-up. We collected data on a large cohort of children and adult thalassemia patients treated with HSCT in a single center, with a follow-up ranging from 1 to 30 years (median, 10 years). We analyzed the outcome of 258 patients (median age 12 years, ranging from 1 to 45 years; 54.3% males) undergoing sibling or unrelated HSCT between 1987 and 2016. Overall, 173 sibling (67.1%) and 85 unrelated procedures (32.9%) were performed; 97 patients (37.6%) were adults (age ≥16 years), whereas 161 were pediatric patients. Pediatric patients were classified according to the Pesaro transplant risk score: 57 patients (22.1%) were in class 1, 83 (32.2%) in class 2 and 21 (8.1%) in the higher risk, class 3. The conditioning regimen was based on Busulfan (BU) in most patients (80.6%), (BU-Cyclophosphamide (CY) in 181 patients; BU-CY-Thiotepa in 27 patients) whereas the conditioning regimen of the remaining patients (19.4%) was based on Treosulfan (Treo-TT-Fludarabine). The graft-*versus*-host disease (GvHD) prophylaxis was based on cyclosporine (CSA) and methylprednisolone, CSA and methotrexate (MTX), or CSA-MTX and anti-lymphocyte globulin (ATG) in the unrelated setting. The 30-year Kaplan-Meier estimates of overall survival (OS) and thalassemia-free survival (TFS) were 85.2% and 77.8%, respectively (Figure). Significantly higher OS and TFS were found in class 1 and 2 patients compared to class 3 patients and adults (OS=95.9% and 93.3% *vs* 74.1% and 68.7%; p<0.001); (TFS=88.3% and 84.2% *vs* 69.8 and 66.3%, p=0.025). The Pesaro risk score with adult class and sibling HSCT variables were significantly associated with OS and TFS in the multivariate analysis. No significant associations between OS, TSF and sex, age at HSCT, donor age or sex, serum ferritin value, hepatitis virus infection, decade of HSCT, Busulfan *vs* Treosulfan-based conditioning regimen emerged. The cumulative probabilities of grade 3-4 acute GVHD and chronic GVHD were 9.9% and 10.5%, respectively. In conclusion, HSCT led to a high rate of cure and survival in this large cohort of thalassemia patients with a very long follow-up.

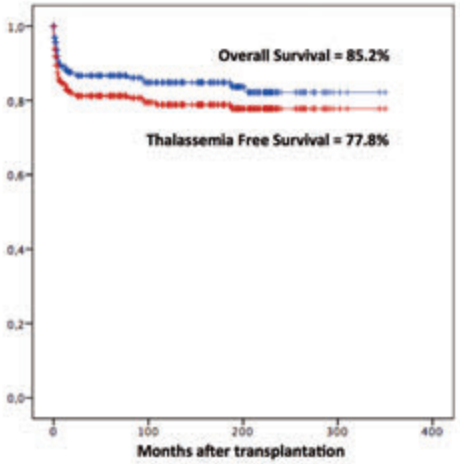

Figure 1.

C038

A PHASE II, MULTICENTRE, RANDOMIZED TRIAL COMPARING BUSULFAN-FLUDARABINE VERSUS THIOTEPA-FLUDARABINE AS A REDUCED-INTENSITY PREPARATIVE REGIMEN FOR ALLOGENEIC HAEMATOPOIETIC STEM-CELL TRANSPLANTATION IN PATIENTS WITH MYELOFIBROSIS

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Allogeneic haematopoietic stem cell transplantation (HSCT) remains the sole curative option for patients with myelofibrosis (MF). Although a spectrum of conditioning regimens has been used, the optimal preparative treatment before HSCT remains to be defined. We did a phase II randomized study at 21 transplant centers comparing the reduced-intensity conditioning (RIC) fludarabine-busulfan (FB) (conventional arm), that had been already tested in the prospective EBMT study (Kroger et al, Blood 2009) with the RIC fludarabine-thiotepa (FT) (experimental arm), that has been widely used in Italy in the last 2 decades (Patriarca

significant in b3a2 (p 0.04), but not in b2a2 subgroup suggesting that the negative impact of b2a2 is not overcome by the use of 2ndGTKI. Pts with DMR lasting ≥2 years were eligible for TKI discontinuation. Among 51 pts (30%) who discontinued b3a2 transcript predicted a higher discontinuation rate compared to b2a2 (41/102,40% *vs* 10/66,15%; p0.0006). In contrast the Sokal score did not predict discontinuation, except in the b2a2 pts with a low Sokal score who had a higher discontinuation rate compared to pts with higher risk (p0.016). *Conclusion:* Different bcr-abl1 transcript could influence the achievement and stability of DMR, independently of other patient's characteristic or type and numbers of TKI treatments. In particular b3a2 was a predictor of a DMR and as a consequence of a higher frequency of TKI discontinuation. In contrast pts with b2a2 more difficulty achieved a sustained response and in this subgroup of pts Sokal score could be useful for predict it.

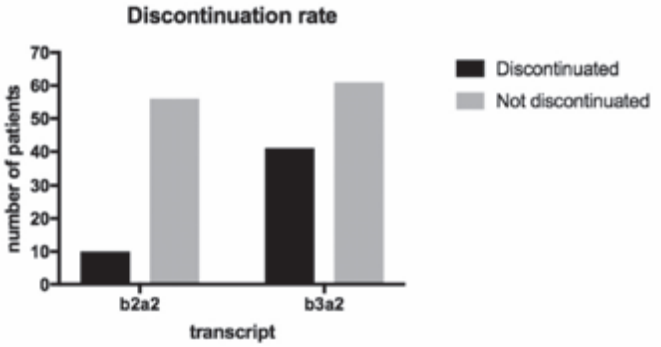

Figure 1. Table with Patients characteristics and graph of discontinuation rate.

Table 1.

Patients characteristics		
	b2a2	b3a2
number of pts	66	102
age	64	66
sokal low	28	41
sokal int	24	38
sokal high	14	23
1^ line therapy		
Imatinib	33 (50%)	61 (60%)
Dasatinib	6 (9%)	7 (7%)
Nilotinib	18 (27%)	23 (22%)
Interferon	9 (14)	11 (11%)
DMR	27 (40.9%)	67 (65.7%)

C035

THE AUTOMATED MOLECULAR TECHNIQUE "ULTRA" ALLOWS A SENSITIVE AND ACCURATE BCR-ABL1 QUANTIFICATION IN PATIENTS AFFECTED BY CHRONIC MYELOID LEUKEMIA

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The correct molecular monitoring of patients affected by chronic myeloid leukemia (CML) is today fundamental for a correct management of patients. The automated methods offer advantages in terms of reduced time for analysis, decreased manual steps, and reduction of possible errors and contamination. We compared the automated technique GeneXpert "Ultra" (Cepheid, Maurens-Scopont, France) with the "Labnet" method; in particular, we compared the sensitivity of the two methods and the consequent classification of patients in molecular response categories. We assessed the BCR-ABL1/ABL1 transcript in 86 patients afferent to laboratories of Pisa, Napoli, Torino, and Bologna (Italy). For statistical analysis, the t-, the Pearson's and the Cohen's K test were adopted. Firstly, we compared the number of detected ABL1 copies, that are fundamental for definition of the molecular response categories, (32,000 for MR4.5, 100,000 for MR5). By the method "Labnet", 51 (81%) samples exceeded the 100,000 copies of ABL1, while by the automated method 81 samples (94.2%) reached >100,000 ABL1 copies. Then, we compared the two methods in discriminating positive and negative samples (K Cohen=0.690; p <0.02): 77 samples were concordant (89.5%) and only 9 (10.4%) were discordant. Of the 18 negative samples with the method "LabNet", 2 (11.1%) were in MR4.0, 10 (55.5%) in MR4.5, and 6 (33.4%) in MR5.0. On the other hand, of the 19 negative samples with the method "Ultra", 1 (5.3%) was in MR4.5 and 18 (94.7%) in MR5.0, confirming the higher sensitivity of the automated method. In the cohort of cases positive by the two methods, the median values of transcript expression were superimposable (p=0.55) and the linear regression coefficient was very satisfying (Pearson's r=0.9399; p-value <0.0001). Finally, we compared the results produced by the two methods according to the "molecular classes" (MR1 *vs* MR2+MR3 *vs* MR4+MR4.5 *vs* MR5). This comparison showed a good concordance between the two methods (Cohen's k=0.78 – good correlation). Variation analysis demonstrated high concordance between "Ultra" and "labnet" methods per EUTOS criteria (Table 1). In a huge series of patients, the automated and manual molecular methods resulted comparable in terms of classification of patients in "molecular classes". The advantage of the "Ultra" technique is represented by the higher number of detected ABL1 copies and the easier standardization.

Table 1.

Category	Sample	Percentage	EUTOS Criteria
Less than 2-Fold Difference	49	77.7%	>50%
Less than 3-Fold Difference	56	88.8%	>75%
Less than 5-Fold Difference	60	95.2%	>90%
Greater than 5-Fold Difference	3	4.8%	NA
Total Samples Analyzed	63	100%	NA

et al, Haematologica 2008). Eligible to this study were patients with primary or secondary MF, age ≤70 years, Karnofsky performance status >60, comorbidity index <5 and with at least one of the following unfavorable prognostic factors: anemia (Hb <10g/dL), leukocytosis ($25x10^9$/L), circulating blasts >1% or constitutional symptoms. Patients were randomized to receive intravenous busulfan 8mg/kg or thiotepa 12mg/kg associated to fludarabine 180mg/mq. Anti-thymocyte immunoglobulin 7mg/kg was administered in case of unrelated donors. From July 2011 to November 2015, 62 patients with a median age of 56 years (36-66) were enrolled. DIPSS score was intermediate-1, intermediate- 2 and high in 21, 38, and 3 patients, respectively. On an intention-to-treat basis, the primary study endpoint was 1 year-PFS. Donors were HLA-identical sibling (25), HLA-matched unrelated (25) or mismatched for a single class I HLA allele (10). At day+30, 52/ 57 patients (91%) engrafted. With a median follow-up of 16 months of patients alive, at 1 year the following outcomes were observed in the FB *vs* the FT arm: PFS was 48% *vs* 69%, [HR 0.60 (95%CI 0.28-1.27) p=0.17], OS was 61% *vs* 80% [HR 0.50 (95%CI 0.21-1.21) p=0.13] and NRM was 27% *vs* 21% (Gray's test p=0.59). Fourteen/50 evaluable patients (28%) developed grade II-IV acute GVHD, that was severe in 4 cases (8%). Mild or moderate chronic GVHD was observed in 7/40 patients (17%). A lower 1-year PFS was observed in patients with DIPSS score high and in patients transplanted from unrelated donors. This prospective randomized phase 2 study showed that FB and FT regimens had a comparable toxicity and disease control at a median follow-up of 16 months for living patients, suggesting that NRM and disease progression remain open issues for MF patients undergoing HSCT, no matter the conditioning regimen.

C039

TRANSPLANT OUTCOME FOR PATIENTS WITH ACQUIRED APLASTIC ANEMIA OVER THE AGE OF 40: MORTALITY HAS NOT BEEN REDUCED IN 2015

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Mortality following HSCT in pts with SAA over the age of 40 is reported to be in the order of 50%; international guidelines recommend first line immunosuppressive therapy above the age of 40. The aim of this study is to assess whether this is still true in 2015. We studied 768 pts from the WPSAA EBMT registry, aged 40 years or more, grafted between 2001 and 2015. Pts were divided in two transplant eras: 2001-2009 (n=329) and 2010-2015 (n=439). In the 2010-2015 period pts were older (52 *vs* 50 years, p<0.01), were more often grafted from alternative donors (ALT) (52% *vs* 28%, p<0.01), with a greater use of BM (53% *vs* 42%, p<0.01), and more often received a fludarabine containing regimen (60% *vs* 42%, p<0.01), and ATG or Campath (87% *vs* 67%, p<0.001). The 5 year OS of patients grafted in 2001-2009 was 61%, compared with 58% for pts grafted in 2010-2015 (p= 0.9). In multivariate analysis including the interval between diagnosis and transplant, the patient's age, donor type, stem cell source, the use of fludarabine and ATG or Campath, the lack of improved survival in 2010-2015 was confirmed (RR 0.9, 95%CI 0.7-1.19; p=0.5). A very strong age effect was shown both in univariate and multivariate analysis: 5 year OS of patients aged 40-49 years, 50-59 years and >60 years, was respectively 66%, 57%, 40% (p<0.0001). The second favorable predictive factor was the use of either ATG or Campath (RR 0,58- 95%CI 0.43-0.76, p=0.002). Regarding HSCT from HLA identical siblings, we could show identical 5 year survival for pts aged 40-49 years in 2010-2015 as compared to 2001-2009 (67% *vs* 70% p=0.8); for patients aged 50-59 years (59% *vs* 60%, p=0.9) and patients over 60 (47% *vs* 50%, p=0.9). For UD the 5 year survival in pts aged 40-49 was 64% in 2010-2015, *vs* 56% in the earlier period (p=0.1), and 53% *vs* 58% for pts aged 50-59. Only 8 patients over 60 grafted in the 2001-2009 period; 61 patients were grafted in 2010-2015 with a 47% actuarial 5 year survival. Combined primary and secondary graft failure was reduced from 16% to 12% in the two time periods (p=0.02), acute GvHD grade II-IV was reduced from 15% to 11% (p=0.1) and chronic GvHD was also reduced from 32% to 26% (p=0.04. Infections remain the leading cause of death in both transplant eras (18% and 22% respectively), followed by GvHD (5% and 4%) and graft failure (5% and 2%),whereas PTLD have been reduced from 3% to 0,5%. HSCT in pts with acquired SAA over the age of 40 years, continues to carry a significant risk of mortality, also in 2015, ranging from 30% in younger SIB transplants to over 50% in older pts.

C040

ROLE OF GRAFT CELL COMPOSITION AND SOURCE IN HAPLOIDENTICAL TRANSPLANTATION USING POST-TRANSPLANT CYCLOPHOSPHAMIDE

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Graft cell composition and source are known to have a prognostic role in in allogeneic hematopoietic cell transplant (HCT). Currently, there are no data in the setting of haploidentical-HCT with post-transplant cyclophosphamide (PT-Cy). *Patients and methods:* Bone marrow (BM) was used as graft source in 29 patients and peripheral blood stem cell (PBSC) in 122 patients. *Results:* For the whole cohort, neutrophil and platelets engraftment cumulative incidences at day +30 were 92% (95% CI: 86-95) and 63% (95% CI: 54-70), with no differences between BM or PBSC. Grade II-IV and grade III-IV acute GVHD cumulative incidences at day +100 were 38% (95% CI: 30-46) and 16% (95%CI:10-23), respectively. Acute GVHD cumulative incidence was significantly lower for BM grafts [14% (95% CI: 4-29) *vs* 48% (95% CI: 34-53), p<0.01] but no differences were observed regarding grade III-IV acute GVHD [7% (CI 95%: 1-21) *vs* 19% (CI 95%: 12-27), p=0.21]. Chronic GVHD cumulative incidence at 18 months was 23% (95%CI: 15-32) with no difference between BM or PBSC. With a median follow up of 15 months (range 0.8 - 40.6), the 18-month PFS and OS were 48% (95%CI: 38-57) and 63% (95% CI: 53-70), respectively. NRM and RI/POD at 18 months were 18% (95%CI: 11-26) and 38% (95%CI: 27-49).Multivariable analysis was performed using patient (age, patient gender, HCT-CI, DRI) and transplant characteristics (graft source, conditioning intensity). A DRI>1 was the only factor associated with higher RI/POD (HR 1.90, 95% CI: 1.25-2.89, p=0.003), lower PFS (HR 1.61, 95%CI: 1.21-2.14, p<0.01), and lower OS (HR 1.67, 95%CI: 1.17-2.27, p<0.01).Graft source did not affect survival outcomes. For the BM group, univariable analysis showed that CD4 graft count >$20x10^6$/kg (median) was associated with a trend toward prolonged 18-month PFS [63% *vs* 34%, p=0.05]. Among the CD4 subsets analyzed, naïve T cells >$8.5x10^6$/kg (median) and recent thymic emigrants >$6.9x10^6$/kg (median) were the only phenotypes associated with a better PFS and OS (p=0.02 and p=0.04 respectively). For the PBSC group, higher CD3 graft count >$230x10^6$/kg (median) was associated with a higher 18-month cGVHD incidence [14% *vs* 0%, p=0.03]. *Conclusions:* we did not observe significant differences in survival outcomes based on graft source. For the BM group, a higher CD4 count was predictive of better PFS. A negative effect of CD3 cell count relating to a higher cGVHD incidence was observed in the PBSC group.

Myeloma and Monoclonal Gammopathies 1

C041

THE UPDATED IMWG FRAILTY SCORE IDENTIFIES 2 CATEGORIES OF PATIENTS NEW FIT AND FRAIL: ANALYSIS AFTER 5 YEAR FOLLOW-UP

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Background: The IMWG frailty score (Palumbo A Blood 2015) that combines age and geriatric assessment (GA) predict survival and toxicity in elderly newly diagnosed MM (NDMM) patients (pts). This analysis is updated after a follow-up of almost 5 years. *Methods:* Pts with NDMM, ineligible for ASCT, enrolled in 3 trials (Magarotto V Blood 2015; Larocca A Leukemia 2016; Bringhen S Blood 2014), were analyzed. At diagnosis, a GA had been performed, to assess comorbidities, cognitive and physical status.

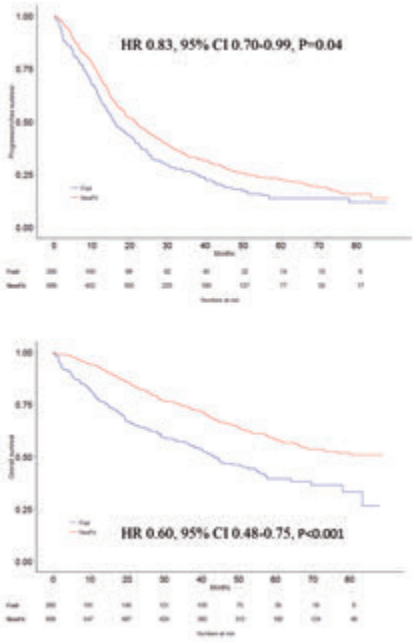

Figure 1. New Fit and Frail patients.

Results: 869 pts (659 received lenalidomide, 152 bortezomib, 58 carfilzomib-based therapy) were analysed. Median age was 74 years (46% ≥75 years and 19% ≥80 years). Updated median follow-up was 57 months. Based on the frailty score, 3 groups were identified: fit (score=0, 39%), intermediate (score=1, 31%), and frail (score≥2, 30%). 52% intermediate and 100% frail pts received reduced-dose therapy according to protocol (age>75 years). 93% fit pts received full dose treatment. Intermediate pts showed a similar TTP (median 20.9 *vs* 20.5 months; HR 0.97, P=0.80), a slightly higher PFS (18.8 *vs* 15.7 months; HR 0.88, P=0.20) and a significantly higher PFS2 (48.4 *vs* 31.8 months; HR 0.72, P=0.006) and OS (72.8 *vs* 43.8 months; HR 0.60, P<0.001) as compared to frail pts. Notably, the PFS2 and OS of intermediate was comparable to fit pts (44.6 months and NR). Since most intermediate pts received reduced-dose treatments (like frail pts), probably they had a higher risk of progression (comparable to frail) due to under-treatment. However 2nd line therapy could be able to rescue intermediate pts producing an improved long-term outcome (comparable to fit), whereas toxicity from 1st line treatment had probably precluded an effective 2nd line therapy for frail pts. Therefore, the intermediate pts can be considered fit (showing a similar PFS-2 and OS) and a new definition can be proposed: new fit (score 0-1, 70%) and frail pts (score ≥2, 30%). The median PFS was 21.5 *vs* 15.7 (HR 0.83, P=0.04), and OS was NR *vs* 43.8 months (HR 0.60, P<0.001) in new fit and frail pts, respectively. *Conclusions:* In this updated analysis the intermediate group is removed and is included in the category of new fit. It is essential to identify frail pts that had a higher risk of progression/death compared to new fit, highlighting the need for specific tailored strategies for frail pts to optimize tolerability and efficacy.

C042

A PHASE 1-2 STUDY OF CARFILZOMIB-POMALIDOMIDE-DEXAMETHASONE IN PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE

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Background: Patients (pts) with relapsed/refractory myeloma (RRMM) have a poor outcome. Pomalidomide and Carfilzomib monotherapy only slightly improved survival. Once- and twice-weekly Carfilzomib showed similar efficacy. Here, we assessed weekly Carfilzomib, Pomalidomide and Dexamethasone for RRMM. *Methods:* in the phase 1, the primary objective was to define the maximum tolerated dose (MTD) of Carfilzomib; in the phase 2 to evaluate response rate. Pts who received 1-3 prior lines of treatment and were refractory to Lenalidomide were eligible. Treatment consisted of 28-day cycles of Pomalidomide 4 mg d 1-21 (1 week off), Dexamethasone 40 mg once-weekly and Carfilzomib at escalating doses (from 36 to 54 mg/m^2) d 1,8,15 until relapse/intolerance. *Results:* 57 pts were enrolled. Median age was 62 years, median time from diagnosis was 3.7 years. 15 pts were enrolled in the phase 1. The first 3 pts received Carfilzomib 36mg/m^2 and had no DLT. In the next cohort with Carfilzomib 45mg/m^2, 1 G3 hypertension and 1 sudden death occurred. Another 3 pts were enrolled at 36mg/m^2: 1 had a G3 atrial fibrillation, 1 G3 hypertension and 1 G5 heart failure. Both the sudden death and the heart failure were preceded by an hypertensive crisis. New procedures were then established by a safety committee to evaluate cardiac function of potentially eligible pts, including 24h blood pressure monitoring before enrolment and serial measurement of blood pressure during treatment. Six more pts were enrolled at 27mg/m^2, none had a DLT. The MTD was 27mg/m^2. 42 pts were enrolled in the phase 2, thus 48 received the recommended phase 2 dose (RP2D). In RP2D pts, neutropenia (65%), thrombocytopenia (12%), infection (8%) and fatigue/asthenia (9%) were the most frequent drug related G≥3 AEs. All G cardiologic AEs were 19%, G3-4 were 8%. Implementation of the new procedures significantly reduced cardiac AEs (all G, G3-4, hypertension and major events) with no new fatal events. ORR was 64%, with 26% ≥VGPR and 6% nCR/CR. After a median follow-up of 13.6 months, median PFS was 9.2 months and median OS was not reached. *Conclusions:* This is the first phase 1-2 trial combining weekly Carfilzomib, Pomalidomide and Dexamethasone. The combination was highly effective, it doubled median PFS in comparison with Pomalidomide or Carfilzomib alone. A simple and careful cardiologic assessment before treatment significantly reduced cardiac AEs.

C043

UPFRONT SINGLE *VERSUS* DOUBLE AUTOLOGOUS STEM CELL TRANSPLANTATION FOR NEWLY DIAGNOSED MULTIPLE MYELOMA: A MULTICENTER, PHASE III STUDY OF THE EUROPEAN MYELOMA NETWORK (EMN02/HO95 MM TRIAL)

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Prospective comparison of single *vs* double autologous stem cell transplantation (ASCT) for the treatment of newly diagnosed multiple myeloma (MM) is needed in the novel agent era. The phase III EMN02/HO95 study was designed to randomize (R1) patients (pts) to standard-dose intensification therapy with bortezomib-melphalan-prednisone (VMP) *vs* high-dose intensification therapy with melphalan (200 mg/sm, HDM) followed by ASCT. In centers with a policy of double ASCT, pts were randomized in a 1:1:1 ratio to receive either VMP or single ASCT (ASCT-1) or two sequential courses of HDM and double ASCT (ASCT-2). A second randomization to consolidation therapy *vs* no consolidation was planned after intensification, to be followed by lenalidomide maintenance in both arms. Aim of the present analysis was to prospectively assess the efficacy of ASCT-1 *vs* ASCT-2. According to study design, 614 pts were randomly assigned to either VMP (n=199) or ASCT-1 (n=208) or ASCT-2 (n=207). Only pts randomized to ASCT-1 or ASCT-2 were included in the current analysis. No significant differences were detected in the baseline pts characteristics in the two groups. The frequency of ISS stage III was 18% in the ASCT-1 group and 19% in the ASCT-2 group, while revised ISS stage III was 9% and 11%, respectively. A high-risk (HR) cytogenetic profile, defined by presence at FISH analysis of t(4;14) and/or del(17p) and/or t(14;16), was observed in 26% and in 21% of evaluable pts, respectively. Median follow-up from R1 was 32 (IQR:26-41) months. On an intention-to-treat basis, 3-year estimates of PFS were 62% in the ASCT-1 arm and 74% in the ASCT-2 arm (HR=0.7; 95% CI=0.49-1.0; P=0.05). PFS benefit with ASCT-2 was retained across predefined subgroups, including pts with LDH values above the upper limits (HR=051; CI=0.27-094; P=0.03), revised ISS stage II (HR=0.54; CI=0.35-0.84; p=0.007), and HR cytogenetics (HR=0.49; CI=0.24-0.98; P=0.046). In a multivariate Cox regression analysis, randomization to ASCT-2 (HR=0.65; CI=0.43-0.96; P=0.032) emerged as the leading independent predictor of PFS. Overall survival was not yet mature and no difference between the two groups was evident. Upfront double ASCT after bortezomib-based induction therapy for NDMM was superior over a single ASCT in terms of prolonged PFS. Clinical benefits with double ASCT were mostly seen in high-risk pts, in particular in those with unfavourable cytogenetics.

C044

INTENSIFICATION THERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION *VERSUS* BORTEZOMIB-MELPHALAN-PREDNISONE FOR NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS: A MULTICENTER, PHASE III STUDY OF THE EUROPEAN MYELOMA NETWORK (EMN02/HO95 MM TRIAL)

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The prospective, multicenter, phase III EMN02/HO95 MM trial was designed to randomly compare (R1) (1:1 ratio; stratification by ISS) four 42-day cycles of standard dose bortezomib-melphalan-prednisone (VMP) *vs* either a single or two sequential courses of melphalan 200 mg/m^2 followed by autologous stem cell transplantation (ASCT), as intensification therapy for newly diagnosed multiple myeloma (MM) patients. A second randomization (R2) to consolidation therapy with bortezomib-lenalidomide-dexamethasone *vs* no consolidation was performed after intensification, to be followed by lenalidomide maintenance until progression or toxicity in both arms. A primary study end point was progression-free survival (PFS) from R1. From February 2011 to April 2014, 1510 patients aged ≤65 years were registered. Of these, 1192 patients randomized to VMP (n=497) or ASCT (n=695) could be evaluated after having received 3 to 4 cycles of bortezomib-cyclophosphamide-dexamethasone induction therapy. Median age was 58 years in both groups, ISS stage III was 21% in VMP and 20% in ASCT arms, while revised ISS stage III was 9% in both groups. The frequency of conventionally defined high-risk cytogenetic changes, including t(4;14) and/or del(17p) and/or t(14;16) as detected by FISH analysis, was 25% in both groups. Median follow-up from R1 was 32 (IQR 25-40) months. On an intention-to-treat basis, median PFS was 42.5 months in the VMP arm and was not yet reached in the ASCT arm; 3-year estimates of PFS were 57% and 65%, respectively (HR=0.73; 95% CI=0.61-0.88; P=.001). PFS benefit with ASCT was retained across predefined subgroups, including patients with a high-risk cytogenetic profile (HR=0.53; CI=0.37-0.76; P=0.001). The probability of achieving a very good partial response or higher quality response was 85.5% in the ASCT group *vs* 74% in the VMP group (odds ratio=1.90; CI=1.42-2.54; P<0.001). In a multivariate Cox regression analysis, randomization to ASCT (HR=0.69; CI=0.55-0.86; P<0.001) and absence of high-risk cytogenetic abnormalities (0.69; CI=0.52-0.91; P=0.008) were the leading independent predictors of prolonged PFS. Overall survival was not yet mature and no difference between the two treatment groups was evident. It is concluded that upfront ASCT still continues to be the reference treatment choice for fit patients with newly diagnosed MM, even in the era of bortezomib-based therapies.

C045

NEOD001 DEMONSTRATES ORGAN BIOMARKER RESPONSES IN PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS AND PERSISTENT ORGAN DYSFUNCTION INDEPENDENTLY OF PREVIOUS HEMATOLOGIC RESPONSE

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Introduction: Light chain (AL) amyloidosis is a rare and often fatal disease caused by the accumulation of misfolded light chains (LCs). Amyloid can lead to progressive failure of critical organs and systems causing significant morbidity and mortality. Current therapies limit LC production; however, ~75% of patients have persistent organ dysfunction. NEOD001 is a novel investigational antibody thought to neutralize circulating LC aggregates and clear insoluble deposits. Here we assessed safety and tolerability, and analyzed organ responses based on consensus criteria and the association between organ responses and previous plasma cell–directed (PCD) treatment. *Patients and Methods:* Inclusion criteria for this trial were that patients complete ≥1 PCD treatment before enrollment, attain partial hematologic response (HR) or better to any previous therapy, and have persistent organ dysfunction. NEOD001 was administered intravenously every 28 days. During the dose-escalation phase, 27 patients received NEOD001 at 0.5, 1, 2, 4, 8, 16, or 24mg/kg in a 3+3 study design. An additional 42 patients with renal, cardiac, or nerve involvement were enrolled and treated (24mg/kg) in the expansion phase. *Results:* In the overall population (N=69), the median age was 61 years (61% male). Median (range) time since diagnosis was 2.9 (0.4-16.0) years. Median time since last PCD treatment to the start of NEOD001 intervention was 5.8 (range, 0.6-85.8) months. NEOD001 treatment was not associated with dose-limiting toxicities, discontinuations, antidrug antibody development, or treatment-related serious adverse events. The study included 36 cardiac, 36 renal and 11 PNS-evaluable patients. Best response rate indicating organ response was observed in 53% of cardiac-evaluable patients (n=19/36), 64% of renal-evaluable patients (n=23/36) and 82% of PNS-evaluable patients (n=9/11). Patients' observed organ response was not related to time from or depth of patients' previous best HR to PCD treatment. Similarly, time or type of patients' last HR did not impact NEOD001 organ response rate. *Conclusions:* NEOD001 is a first-in-class antibody that specifically targets disease-causing, misfolded light chain aggregates in AL amyloidosis. These results demonstrated that NEOD001 infusions were safe, well tolerated and associated with organ responses independently of time since previous chemotherapy, depth of hematologic response, or predominant type of PCD treatment.

Cytogenetics and Laboratory Investigation

C046

TARGETED LOCUS AMPLIFICATION (TLA) TO IDENTIFY NOVEL, TRANSLOCATION-BASED, MOLECULAR MARKERS FOR MINIMAL RESIDUAL DISEASE (MRD) MONITORING IN MANTLE CELL AND FOLLICULAR LYMPHOMA

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Minimal residual disease (MRD) monitoring by PCR methods is a strong predictor of clinical outcome in mantle cell (MCL) and follicular (FL) lymphoma. However, the identification of a molecular marker is possible only in 80% of MCL and 60% of FL patients. Next generation sequencing (NGS) tools might overcome these limitations. Indeed, the recently developed targeted locus amplification (TLA) technology, based on the crosslinking of physically proximal DNA loci, was able to identify novel candidate oncogenes in acute lymphoblastic leukemia. TLA approach was tested in highly infiltrated MCL and FL baseline samples, with at least one translocation confirmed by FISH but a molecular marker identification failure, with the aim to increase the rate of success in marker screening for MRD purposes. Moreover, the performances of the newly identified molecular markers were compared to the standardized IGH qPCR approach for MRD monitoring. Genomic DNA was extracted from BM or PB samples of MCL and FL patients, enrolled in prospective clinical trials of the Fondazione Italiana Linfomi (FIL). Libraries for TLA (Cergentis, Utrecht) were prepared using primers targeting the IGH-enhancer locus, and sequenced on Illumina platform. The new breakpoint sequences obtained was validated by ASO qPCR. Finally, the efficiency of TLA novel markers to track MRD was compared to MRD data obtained from clonal IGH rearrangements previously detected and tracked by standardized ASO qPCR approaches, following the EuroMRD guidelines. TLA was firstly tested on 17 t(11;14)-positive, BCL-1/IGH MTC-negative MCL baseline samples identifying in all the cases a novel BCL-1/IGH breakpoint. Therefore, additional 5 t(14;18)-positive, BCL-2/IGH MBR/mcr/"minor"-negative FL BM samples were tested, with again a 100% success rate. In addition, the MBR-positive control sample (DOHH2 cell line) was correctly sequenced, as well. Secondly, ASO primers were designed on the newly identified BCL-1/IGH "minor" breakpoints and MRD was monitored by qPCR: in 13 cases where also IGH-based marker was available, MRD results were highly comparable between the two markers (Figure 1), with good and overlapping overall correlation (r2=0,86). ASO qPCR FL cases is ongoing. The TLA NGS technology allowed to identify a novel molecular marker in FISH-positive MCL and FL baseline samples, where the classical Sanger sequencing failed. These new breakpoints can reliably be used for MRD detection in previously not traceable patients.

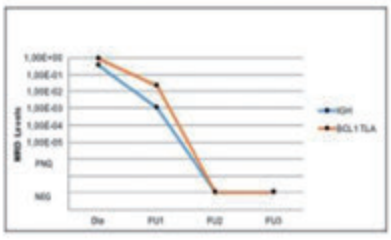

Fig 1: MRD was monitored using IGH rearrangement (blue) and BCL1/IGH translocation (orange). The results showed as the BCL1/IGH sequence obtained by TLA was useful in MRD monitoring as IGH rearrangement detected using classic sequencing techniques. Moreover, in FU1 samples tumour burden detected by TLA BCL1/IGH was higher than IGH results.

Figure 1.

C047

MUTATIONAL ANALYSIS IN BCR-ABL1 POSITIVE LEUKEMIA BY DEEP SEQUENCING BASED ON NANOPORE MINION TECHNOLOGY

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In newly-diagnosed chronic phase (CP)-CML patients, 15–30% who start first-line tyrosine kinase inhibitors (TKIs) therapy will not reach an optimal response, and a BCR-ABL1 kinase domain (KD) mutation will be detectable in 25-50% of patients with treatment failure with an increased frequency of these mutations observed in accelerated phase and blast crisis patients. Sanger sequencing (SS) is considered the gold standard for mutation detection with a sensitivity of around 20% of allelic ratio. Recently next generation sequencing (NGS)-based assays have been reported for detecting BCR-ABL1 KD mutations; although these NGS strategies are more sensitive than SS, they are burdened by costs related to the initial investment and the required reagents. MinION is a single molecule sequencer connected to a laptop through a USB3.0 interface, based on nanopore technology. We describe a third-generation sequencing assay on MinON for detecting BCR-ABL1 KD mutations and compare the results to a SS-based test in 24 Ph+ leukemia cases. Among them, 12 (11 CML and 1 ALL cases) developed treatment resistance during the TKI's treatment course (Group 1) and 12 were at diagnosis (7 CML and 5 ALL cases) (Group 2). All cases included in the study were analyzed by SS and MinION sequencing in blinded manner. Two sequencing runs were performed with the two different pools of patients: the first lasted eight hours and was carried out on the Group 1, whereas the second run included the Group 2 and lasted 24 hours to achieve a deeper sequencing. Sequencing results showed that ABL1 was covered from exon 2 to 10 and that the mean of the sequencing depth was around 150x and 1000x for Group 1 and 2, respectively. We found 10 BCR-ABL1 KD mutations in 9 patients belonging to the Group 1 (one case showed compound mutations), generally with high allelic ratio. MinION data analysis on the Group 2 was able to detect mutation only in a ALL case. Results from MinION and SS showed 92% concordance in all cases included in this study. Moreover, two mutations that were initially undetectable by SS became evident thanks to the indications coming from MinION analysis. Our findings demonstrate that MinION is suitable for employment in hematology laboratory for detecting BCR-ABL1 KD mutation in Ph+ leukemias and that the main advantage of this technology is to allow a more efficient and sensitive analysis than SS at very competitive costs.

C048

THE IMPACT OF GENETIC POLYMORPHISMS ON R-CHOP EFFICACY IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: AN INTERIM ANALYSIS OF A MULTICENTER PROSPECTIVE PHARMACOGENETIC STUDY

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Introduction: Chemoimmunotherapy with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) is the current standard of care for patients (pts) with previously untreated diffuse large B-cell lymphoma (DLBCL). However, lack of remission or early relapse remains a major clinical issue in DLBCL, with 30% of pts failing standard of care. Although clinical factors and molecular signatures can

partially predict DLBCL outcome, additional information is needed to identify high-risk pts, particularly biologic factors that might ultimately be amenable to intervention. Thus, we designed a multicenter prospective trial aimed to identify gene polymorphisms that may affect response and tolerability to R-CHOP. We report the results of an interim analysis of the first 80 enrolled pts. *Methods:* This study enrolled pts with newly diagnosed DLBCL, candidates for R-CHOP chemotherapy. Genomic DNA was extracted from peripheral blood of 80 pts. Single nucleotide polymorphisms (SNPs) analysis was performed by an Affimetrix array. To date, 21 SNPs from 19 candidate genes (ABCB1, ABCC1, ABCC2, ABCG2, CYBA, CYP2C9, FCGR2A, GSTP1, IL2, MARCKS, MLH1, NCF4, NQO1, NQO2, RAC2, TNF, TOP2A, TP53, TUBB) involved in pharmacokinetics and pharmacodynamics of R-CHOP were analysed. At the planned interim analysis, the impact of SNPs on objective response rate, progression free survival (PFS) and overall survival (OS) was evaluated. *Results:* Median age was 63 years with 47% male. At baseline, according to R-IPI, 26% were poor prognostic group, 59% good and 15% very good prognostic group. The median number of treatment cycles was 5.85 (range: 4-6). At the end of treatment, complete response was achieved in 89% and a partial response in 6% pts. Multivariate analysis identified FCGR2A rs1801274 as a predictor of PFS (p=0.045). Pts with HR or RR genotypes showed shorter PFS than pts with HH genotype (HR: 2.437, CI95% 1.020-5,823). No statistically significant correlation was found between SNPs and OS. *Conclusions:* The preliminary data obtained in a limited number of pts, show an association between a SNP of the low affinity FCGR2A gene involved in the activity of rituximab and PFS. Further data will be available after completion of pts accrual at the end of the study. Acknowledgements This study was supported by a grant from the Associazione Giacomo Onlus, Castiglioncello (LI), Italy to E.M. and Cassa di Risparmio di Firenze, Firenze, Italy to S.N.

C049

FLOW-CYTOMETRIC ASSESMENT OF LYMPH NODE SUSPENSIONS WITH CLINCAL SUSPICION OF NON-HODGKIN LYMPHOMA. A SINGLE CENTER EXPERIENCE

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Background: Few studies have addressed the utility of flow cytometry on tissue samples of lymph nodes. A high concordance between results of flow cytometry and immunohistochemistry has been reported in a single large series of patients with non-Hodgkin lymphoma (NHL). *Aim:* We report our experience with a flow-cytometric diagnostic approach to lymph node tissue in patients with a clinical suspicion of lymphoproliferative disorders. *Methods:* Lymph node suspensions were prepared by mechanical disaggregation of the of solid tissue using the Medimachine system (Becton Dickinson). The screening panel included CD19, CD20, CD10, Kappa, Lambda, CD5, CD3, CD4 and CD8. Results of immunophenotyping were compared with immunohistochemical diagnoses. *Results:* We assessed 33 lymph node suspensions by flow cytometry. Immunohisotchemical diagnoses were diffuse large B-cell lymphoma (DLBCL) in 10 patients, follicular lymphoma (FL) in 4 patients, marginal zone lymphoma (MZL) in 1 patient, Hodgkin lymphoma (HL) in 8 patients, T-cell lymphoblastic lymphoma (T-ALL) in 1 patient, mycosis fungoides (MF) in 1 patient, metastatic carcinoma in 4 patients, reactive lymph node changes of various types in 4 patients. All B-subtype non-Hodgkin lymphomas (B-NHL) were correctly identified (100% sensitivity) by flow-cytometric analysis. B-cell pathological populations were CD20 positive and presented a clear clonal light chain restriction in 9 cases out of 15 (6 DLBCL and 3 FL), while 6 cases (4 DLBCL, 1 FL and 1 MZL) did not show any light chain expression. All DLBCL cases displayed an aberrant population with high forward and side scatters, while the 4 FL and the MZL cases showed low scatter parameters. CD10 was expressed in all FL cases and in 2 out of 10 DLBCL (20%). CD5 was present in 1 case of DLBCL (10%). In non B-NHL samples, we observed a physiological B-cell population with a normal kappa/lambda ratio. We detected a pathological T-cell population with co-expression of CD4 and CD8 and CD3 dim expression in the sample of T-ALL patient. Moreover, lymph node suspension of MF presented the typical phenotype CD4+/CD26-/CD7±. *Conclusions:* In our limited series of patients, flow-cytometric assessment of lymph node suspensions had a high sensitivity and specificity for B-NHL. It could be useful to provide the clinician and pathologist with rapid information to guide further diagnostic work-up and direct therapy in symptomatic patients with bulky disease.

C050

GENOMIC PROFILE ANALYSIS IN MUTLIPLE MYELOMA: COMPARISON OF FISH VS SNP ARRAYS PROCEDURE

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FISH is considered the gold standard for the detection of genomic aberrations, which characterize virtually every Multiple Myeloma (MM) patient; however, SNP arrays represents a technology mature enough, to implement FISH for first-line screening in MM. Moreover, SNP arrays might assess the sub-clonal fraction of prognostically relevant chromosomal aberrations, eventually changing throughout the disease course, according to the evolutionary dynamics of MM progression. Aim of the study was to compare FISH and SNP arrays techniques potentialities in the detection of genomic alterations in MM purified CD138+ plasma cells (PC) for diagnostic purposes. Four prognostically relevant genomic alterations were selected to perform this comparison, *i.e.* deletion 1p on CDKN2C (del1p), amplification 1q on CKS1B (amp1q), deletion 17p on TP53 (del17p) and deletion chr13 on RB1 (del13q). CD138+ PC samples were obtained from 51 newly diagnosed MM patients (pts) consecutively collected in our Institution. Purity of PC samples ranged from 30% to 97% (median 83%). For FISH analysis, a total of 250 cells were analyzed, in order to detect genomic alterations (positivity cut-off=10%). Data originated from SNP arrays (Affymetrix 6.0) were analyzed by ChAS v3.1 software; in order to correctly call any given Copy Number Alteration (CNA), all the following parameters were evaluated: CN state, log2 ratio, smooth signal (SS), allele difference and loss of heterozygosity (LOH); in particular, SS was employed to detect imbalances present in subclones and to assess mosaics. Only CEL files with an MAPD >.35 were included in this analysis. In 96% of cases, both del13q and amp1q were concordantly detected, likely due to their clonal nature (median SS 82% and 71%, respectively). On the contrary, more discrepancies emerged with respect to both amp1p and del17p, where 88% and 75% of FISH positive cases, respectively, were concordantly detected. Among discordant cases, most (75% and 80% for del1p and del17p, respectively) were sub-clonal and therefore likely not detectable by FISH. SNP array represent a highly reliable technique candidate to be included in the diagnostic procedures of MM patients, particularly due to the possibility to explore patients genome as a whole and to study several prognostic factors at the same time. However, consistent cut-off values need to be set, in order to determine both the presence of a given alteration and its subclonal nature.

Hodgkin Lymphomas

C051

REAL LIFE EXPERIENCE WITH BRENTUXIMAB VEDOTIN: THE ITALIAN STUDY ON 234 RELAPSED/REFRACTORY HODGKIN'S LYMPHOMA

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Data from phase III studies may not be sufficient to determine the whole description of a drug in routine use and observational studies can be an objective way of mapping risks in the real life. A large Italian multicenter observational retrospective study was conducted on the use of brentuximab vedotin (BV) for patients with relapsed Hodgkin's lymphoma (HL) in the everyday clinical practice according to an Italian national law (Law 648/96: "medicinal products that are provided free of charge on the national health service" active for BV from November 2012 to July 2014") to check if clinical trial results are confirmed even in a real life context. Primary endpoint was the best response; secondary endpoints were the overall response rate at the end of the treatment, duration of response, survival and the safety profile. 234 CD30+ HL patients were treated. Best response was observed after a median of 4 cycles in 140 patients (59.8%): 74 (31.6%) patients obtained a complete response (CR) and 66 (28.2%) achieved a partial response (PR); overall response rate at the end of the treatment was 48.3% (62 CR and 51 PR). The best response rate was higher in the elderly subset (>60 years): 14 (50%) CR and 5 (17.8%) PR. Disease free survival was 26.3% at 3 years and progression free survival 31.9% at 4.5 years. We identified 30 long term responders (patients with a response ≥12 months) of whom 18 are still in CR, 7 with a consolidative transplant and 11 without any consolidative procedure. Duration of response did not differ for who achieved at least PR and then either did or did not undergo consolidative transplant. The treatment was well tolerated and the toxicity profile was closely similar to the previously published data; no death has been linked to BV-induced toxicity. The results of this large retrospective study of 234 relapse/refractory HL in the daily practice support the BV efficacy with manageable toxicity superimposable to the one reported in clinical trials results; in particular, there is the confirmation of the similar activity in different settings, *e.g.* in elderly patients, and of the response duration independently by the transplant consolidation. The relevance of the CR status after 4 cycles and the role of BV as a bridge to transplant for the chemorefractory patients were also pointed up.

C052

HISTOLOGICAL VERIFICATION OF POSITIVE POSITRON EMISSION TOMOGRAPHY FINDINGS DURING THE FOLLOW-UP OF PATIENTS WITH MEDIASTINAL LYMPHOMA: LARGE EXPERIENCE ON 96 PATIENTS

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An important field of application of positron emission tomography (PET) is in the medium and long-term follow-up after complete response of Hodgkin lymphoma (HL) and aggressive non-Hodgkin lymphoma (NHL) with mediastinal involvement at diagnosis. The aim of this study was to verify the reliability of positive PET scans of the mediastinum in following up patients with mediastinal lymphoma, using histological findings as comparison (gold standard). From January 2002 to February 2016, 483 patients with mediastinal lymphoma were followed after the end of front-line treatment. Ninety-six patients with a positive PET scan of the mediastinum underwent computed tomography scanning and surgical biopsy. For 67 HL and 29 NHL a suspicion of lymphoma relapse was raised based on positive mediastinal PET scanning. Histology confirmed relapse in 63 (48 HL and 15 NHL) out of 96 patients (65.6%). In the remaining 33 (34,4%) cases, biopsy revealed: necrotic tissue in 7 patients, fibrosis in 7 patients, thymus in 7 patients, sarcoidosis in 4 patients, tuberculous granulomas in 2 patients, sarcoid-like lymph node granulomatosis in 1 patient, tuberculosis lymph node granulomatosis in 1 patient, reactive inflammation lymph node in 3 patients, and thymoma in 1 patient. The maximum standardized uptake value was significantly higher among patients who had signs of relapse (63 true positive cases) than among those who stayed in remission (33 false positive cases), the median values being 10.30 (range, 3.2-25.0) and 5.0 (range, 2.8-12.6) respectively ($p<0.05$). The analysis on this large series of 96 patients confirms the concept that patients with positive PET in the mediastinum during the follow-up cannot be considered sufficient for final diagnostic purposes considering that at least one third of the patients can present only benign or, anyway, unrelated neoplastic pictures. Histological confirmation can be safely obtained by various biopsy techniques, the choice of which should be made on the basis of the clinical and imaging study findings case by case.

C053

SECOND MALIGNANCIES AND CARDIOVASCULAR DISEASES IN HODGKIN LYMPHOMA SURVIVORS TREATED AT ISTITUTO NAZIONALE TUMORI OF MILAN

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Background: Although it is well known that survivors of Hodgkin Lymphoma (S-HL) are at risk of late effects from chemo- (CT) and radiotherapy (RT), structured survivorship care is still limited and most data on iatrogenic late effects derive from registries or self-reported interviews. *Aims:* Adult S-HL in complete remission for ≥5 years from end of treatment (EOT) were identified and offered a tailored follow-up visit in our survivorship clinic (SC), in order to evaluate the incidence of late complications, like second malignancies (SMs) and cardiovascular diseases (CVDs). *Methods:* Medical records of adult S-HL, treated after 1970, with current age ≤75 years, who underwent a visit at SC, were analyzed to evaluate the incidence of SMs and CVDs. Results were correlated to treatment modalities and descriptive statistics were calculated. *Results:* From May 2014 to January 2017, 333 S-HL (145

males, 188 females), with a median age at HL diagnosis of 32 years, underwent a routine visit at SC. Forty-two percent had been treated for stage I-II A and 58% for stage IIB, III or IV HL. The majority of patients (76%) had received CT+RT, 16% CT and 8% RT only. After a median of 227 months (range, 6–489) from EOT, 50 S-HL (15%) developed SMs with 8 patients diagnosed with ≥1 cancer. The following cancers were observed: breast in 24 patients (7%), bladder/kidney/prostate in 10 (3%), thyroid in 6 (2%), colon in 3 (1%), lung in 2 (0.6%), melanoma in 2 (0.6%), soft tissue sarcoma in 2 (0.6%), non HL in 5 (1.5%) and acute promyelocytic leukemia in 1 patient. Among S-HL developing breast cancer, 96% had previously received ≥30 Gy mantle (14) or mediastinum field RT (9). Previous neck RT was delivered to 5/6 S-HL with thyroid cancer. Furthermore, 29 patients (9%) developed basal cell carcinoma (multiple in 15 cases). CVDs occurred in 104 S-HL (31%) at a median of 13.5 years from EOT and comprised: 24 coronary artery disease (14 myocardial infarction), 13 heart failure, 46 moderate or severe valvular disease and 6 cerebrovascular disease. Valvular disease was strongly associated with RT, as 85% of affected patients had previously received mediastinal RT. *Conclusions:* Results of this study confirm that a substantial proportion of patients face one or more serious late complications of HL treatment and specific health resources for tailored follow-up programs are warranted to improve health in adult HL survivors.

C054

CD30+ GERMINAL CENTER (GC) B CELLS ARE TRANSCRIPTIONALLY RELATED TO LIGHT ZONE GC B CELLS REENTERING THE DARK ZONE, AND TO TUMOR CELLS OF CLASSICAL HODGKIN LYMPHOMA (CHL)

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Rare B cells in GCs and extrafollicular regions of secondary lymphoid tissues express the activation marker CD30, a diagnostic hallmark of cHL. The specific features of these cells and their relation to CD30+ Hodgkin and Reed/Sternberg (HRS) cells of cHL are unclear. Thus, we subjected normal human tonsils (n=17 donors) to MACS/FACS sorting of CD30+ GC B cells (CD30+/CD20hi/CD38+) and CD30+ non-GC B cells (CD30+/CD20+/CD38-), followed by: i) further immunophenotyping for the immunoglobulin heavy chain isotypes and for the GC/post-GC B-cell marker CD27 (n=12 donors); ii) mutation analysis of the immunoglobulin variable (IgV) gene rearrangements (n=4 donors); and iii) microarray-based genome-wide expression profiling (n=5 donors), in comparison to other mature B-cell subsets (5-10 donors for each subset) and to HRS cells microdissected from frozen lymph node biopsies of cHL (n=16 cases). CD30+ B cells represented a tiny population (mean 0.7%, range 0.1-1.7%) of tonsil mononuclear cells, and included a variable fraction of cells expressing GC markers (mean 58%, range 18%-86%). Immunophenotypic and IgV gene analyses indicated that CD30+ GC B cells are typical GC B cells, and that CD30+ non-GC B cells are mostly post-GC B cells. Yet, transcriptionally the two CD30+ subsets were distinct from bulk GC B cells and, respectively, from post-GC memory B or plasma cells, and shared a marked proliferative/anabolic signature. A striking feature common to, and exclusive of, both CD30+ subsets was the strong expression of MYC and its target genes. Thus, human CD30+ GC B cells are probably related to the MYC+ GC B cells known in the mouse to play a crucial role in sustaining the GC reaction, as they represent the rare GC B cells positively selected in the light zone to re-enter the dark zone for further proliferation. Conversely, CD30+ non-GC B cells are likely reactivated post-GC memory B cells undergoing proliferation at extrafollicular sites. HRS cells were transcriptionally more similar to both CD30+ B-cell subsets than to bulk GC B cells, suggesting that HRS cells either derive from CD30+ B cells and retain the transcriptional signature of their cell of origin, or acquired a similar activation signature due to neoplastic transformation. In comparison to CD30+ B cells and other lymphomas, HRS cells showed a remarkable downregulation of genes regulating genomic stability and cytokinesis, which may explain the typical genomic instability and multinuclearity of HRS cells.

C055

A PHASE II STUDY WITH BENDAMUSTINE PLUS BRENTUXIMAB VEDOTIN IN HODGKIN'S LYMPHOMA AND CD30+ PERIPHERAL T-CELL LYMPHOMA IN FIRST SALVAGE SETTING: THE BBV REGIMEN

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Novel salvage options inducing high complete response (CR) rates are needed for patients with primary refractory disease, particularly prior to autologous stem cell transplant (ASCT). Brentuximab vedotin (BV) and bendamustine (B) both showed activity in relapsed Hodgkin lymphoma (HL) patients as monotherapy. Their combination in heavily pretreated (BBV regimen) patients with multiply relapsed or refractory HL and anaplastic large cell lymphoma resulted in an overall response rate (ORR) and CR rate of 67% and 19%, respectively. In a second study in which BV+B was used in HL patients with primary refractory disease or at first relapse reported ORR and CR rate of 93% and 73%, respectively. This is a single-arm, open-label, multicenter, phase II clinical trial on the efficacy and safety of the BBV regimen as first salvage therapy in patients with relapsed or refractory HL or peripheral T-cell lymphoma (PTCL). A total of 25 patients with PTCL, and 40 with HL are expected to be enrolled. In the study, intravenous B will be administered at a dose of 90 mg/m^2 on day 1 and 2 and BV will be given intravenously at a total dose of 1.8 mg/kg on day 1 of each 21 days-based cycle, for 6 cycles. All patients achieving a CR can be considered eligible to peripheral blood stem cell mobilization and may proceed to an ASCT at any time after cycle 4. A first protocol amendment introduced and age upper limit (patients must be 18-60 years old) due to safety concerns of the combination in elderly people. The HL cohort has been just closed while the PTCL cohort is still open (only 3/25 patients were enrolled). The steering committee decided to amend the study protocol to include any relapse/refractory PTCL patients (any line of treatment instead of patients with primary relapsed/refractory disease). 21 HL patients had restaging at the end of BBV regimen, of whom 16 had a response leading to an ORR of 76.2% (8 CR). These patients had already the first restaging after ASCT: 14 are still in CR while 2 had relapse. No grade 4 adverse events (AEs) were report, but among grade 3 AEs we point out skin rashes (10%) related to study drugs. Albeit early, these data suggest that the BBV regimen exhibits promising results in a challenging sub-population of HL patients. Emerging data could continue to establish that BBV is an effective approach that could represent a new bridge to ASCT in primary refractory HL and a valid salvage option for relapsed/refractory PTCL.

Quality of Life

C056

FRONT-LINE VASCULAR ACCESS DEVICES IN ACUTE LEUKEMIAS – PERIPHERALLY INSERTED CENTRAL CATHETER (PICC) VERSUS TRADITIONAL CENTRAL VENOUS CATHETER (CVC): A PHASE IV RANDOMIZED TRIAL (NCT02405728)

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PICCs *versus* traditional CVCs as front-line venous access device in patients with acute leukemias undergoing intensive chemotherapy for remission induction were compared (NCT02405728). Primary endpoint is the occurrence of catheter-related bloodstream infections and/or thrombosis. Secondary endpoints are the occurrence of other complications, such as pneumothorax or catheter occlusion, and quality of life. From April 2015 to February 2017, 152 consecutive patients were randomly assigned (1:1) to PICC (Arm A) or traditional CVC (Arm B). All insertions were followed by ultrasonography assessments and chest X-ray. 152 patients (130 AML and 22 ALL) with a median age of 47 years (r. 13-82), were randomized in the two arms. In the Arm A, 76 PICCs were inserted in 76 patients. Double lumen PICCs (5 Fr) were inserted in 70 patients, single lumen PICCs (4 Fr) were inserted in 5 patients, and triple lumen PICC (6 Fr) was inserted in 1 patient. 68 PICCs were inserted in the right basilica vein, 5 PICCs were inserted in the left basilica vein and 3 PICCs were inserted in the left brachial vein. In Arm B, 76 traditional CVCs were inserted by the Seldinger technique in other 76 patients. 45 CVCs were inserted in subclavian vein and 31 CVCs were inserted in internal jugular vein. Overall, the median duration of in-situ catheter placement was 5 months: 6 months (r. 3-12) in the arm A *vs* 3 months (r. 1-10) in the arm B. In the arm A, catheter-related thrombosis occurred in 8 patients (6 basilica veins, 2 brachial veins) and catheter-related bloodstream infections in 4 patients (4 coagulase-negative staphylococci; of them, 2 meticillin-resistants). In the arm B, 20 cases of catheter-related thrombosis (7 subclavian veins, 13 internal jugular veins) and 15 cases of catheter-related bloodstream infections (10 enterobacteriaceae; 5 coagulase-negative staphylococci, and, of them, 3 meticillin-resistants) were observed. Thus, PICCs were significantly associated with fewer major complications than traditional CVCs (thrombosis: 10.5% arm A *vs* 26% arm B, p=0.01 by 2 test; bloodstream infections: 5% arm A *vs* 19% arm B, p= 0.007 by 2 test) (Figure 1). The preliminary observations of this ongoing Phase IV randomized study, focusing on front-line use of central venous access device in a high-risk hematological population, suggest that the use of PICC represents an advance in terms of decrease of complication rate and improvement of quality of life for patients with acute leukemia.

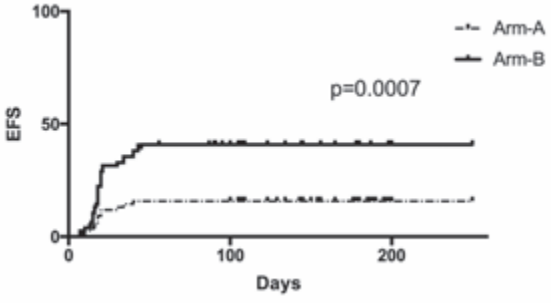

Figure 1.

C057

THE PERCEPTION OF PROTECTIVE ISOLATION IN PATIENTS UNDERGOING HSCT: MULTICENTER STUDY ISOLA15

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Before undergoing hematopoietic stem cell transplantation (HCST), patients receive high doses of chemotherapy, which cause prolonged immunosuppression and enhance the risk of contracting severe infection. Thus, patients usually receive hospital care in protective isolation until full neutrophil recovery. The extent to which the isolation is implemented varies across centers and countries. Strict rules require the placement of a patient alone in a germ-free room with positive pressure, from which the patient cannot leave. However, patients may suffer for being alone in a room, with limited contact with the outside world and with loved ones. This study was aimed at developing a self-report questionnaire to assess the perception of protective isolation from the patient's perspective and testing its psychometric properties. Items of the questionnaire were developed on the basis of a three-dimensional model derived from a metasynthesis. The methodological steps included conceptualisation, a focus group to verify face-validity, item revision and selection on the basis of patient experiences collected in two phenomenological studies, content validity with 22 experts, and cognitive interviews with 5 patients. Overall, 17 items yielded adequate CVI (>0.78). The content validity of the whole scale was 0.88. The dimension "Suffering" was expressed with items about boredom, enhanced fears, feeling imprisoned, movement limitations, feeling cut off from the world, and isolation burden. The dimension "Relating to oneself" was expressed with items about the ability to find meaning, assuming a new perspective, thinking about oneself, finding serenity, and safety. The dimension "Missing the relationship with others" was expressed with items about missing someone to talk with, missing loved ones, feeling detached, and preference for being alone. A validation study was conducted in 10 Italian centres. Participants included 150 adult patients receiving autologous or allogeneic HSCT in protective isolation. Between 7 and 9 days post-transplant, participants were asked to complete the scale about the perception of protective isolation. The scale showed good psychometric properties. Exploratory factor analysis yielded a tri-dimensional model, in line with the metasynthesis. Further studies should confirm these findings and identify risk factors for a negative isolation experience.

C058

PSYCHOLOGICAL DISTRESS SCREENING AND QUALITY OF LIFE IN HIGH RISK OF IPSS MDS PATIENTS: A PILOT STUDY IN ANCONA

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Background: The Screening of distress and Psychological disease in Haematology is not so frequently evaluated, opposed to Medical Oncology. This study is aimed to fill this gap in knowledge and is one of the first trials in Italy with this objective. Methodology 26 MDS patients, 10 females and 16 males (mean age was 67 years (59-81) at high risk of IPSS were evaluated. 22 were treated with subcutaneous azacitidine, 5-2-2 days/ every 28 days and the others received support therapies in our day hospital, The results of Need Valuation Questionnaire, Back Depression Inventory II, Mini Mental Adjustment to Cancer, and Life Expectancy, Severity of Disease and Possibility of Care (Analog Visual Scale) were analysed. *Results:* The needs expressed by the Need Evaluation Questionnaire are: Informative needs 50%; needs related to assistance 10%; relational needs: 19%; psychoemotional Support Needs: 10%; material Needs: 24%. By the analysis of results of Beck Depression Inventory II, somatic (physical) depression is greater than "cognitive" (psychic). The results of the Mini-Mental Adjustment to Cancer Scale suggest high prevalence of fatalism and combat spirit, and also avoidance / minimization. Despair / depression (15%) and anxious concern (19%) are less frequent. The analytical visual scales bring us the following data: 1) The mean data about the perceived quality is 5.84 (range: 1 minor quality-10 higher quality); 2) The mean data about the severity of perceived illness is 4.65 (value 1 most seriously-10 minor gravity); 3) The mean data about the perceived possibility of cure is 4.92 (value 1 minor curability-10 maximum curability): 23% of the patients have a value of 1 or 2 of Quality of Life, (bad); 27% indicates the disease with a severity of 1 or 2 (severe); 23% indicates the illness as difficult to heal (Value 1-2). *Conclusions:* Psychological and emotional discomfort screening is the basis for Structured Psycho-Oncology interventions by Hospital Departments and Day-Hospital Hemology. This pilot study poses the foundations of potentially beneficial psychological interventions to ensure a global care of the hematologic patient.

C059

IMPROVABLE LIFESTYLE FACTORS IN LYMPHOMA SURVIVORS: A MONO-CENTRIC SURVEY

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Background: NCCN and American Cancer Society emphasize the advantages deriving from the promotion of a healthy lifestyle among cancer survivors, particularly in preventing metabolic and cardiovascular sequelae. Recent reports indicate, in fact, that deviation from guidelines is associated with a worse quality of life in non-Hodgkin's lymphoma (NHL) survivors. In the present study, we evaluated different lifestyle factors in a selected population of lymphoma survivors, to identify peculiar targets for an optimal secondary prevention of treatment-related complications. *Patient and Methods:* Since November 2016 to March 2017, we enrolled consecutive patients in continuous remission of lymphoma for at least 3 years and in current follow-up at our Institution. In line with the "CCM2014 Project" supported by the Italian Ministry of Health, lymphoma survivors underwent a survey aimed at analyzing specific lifestyle items according to current international guidelines, including smoking, adherence to the Mediterranean diet (daily intake of more than 3 portions of fruit/vegetables, weekly intake of less than 2 portions of meat), physical activity (at least with a moderate physical activity for 150 minutes a week) and body mass index (BMI). *Results:* 46 patients out of 61 actually enrolled at our Institution, underwent the survey and were eligible for the analysis. Median age at the time of enrollment was 49 years (range 25-78; males n.25, females n.21). Median overall survival and remission time were 8 (range 5-27) and 6.25 years (range 3-26), respectively. 30 patients had a history of Hodgkin's lymphoma (median age at enrollment 44.5 years) and 16 of NHL (median age 56.5). The 91.3% of survivors (n.42) had received an anthracycline-containing regimen, 34.7% (n.16) mediastinal radiotherapy, 8.6% (n.4) an autologous hematopoietic stem cell transplantation. We found that the 19.5% (n.9) had smoking habits, the 78.2% (n.36) was not adherent to the Mediterranean diet and the 80.4% (n.37) had insufficient physical activity. Finally, the 28.2% (n.13) of patients was overweighed and the 26% (n.12) obese. *Conclusions:* The majority of patients showed lifestyle factors considered at risk according to current guidelines. The promotion of educational models of healthy lifestyle appears an emerging need to improve effective actions of secondary prevention and restrain the occurrence of late morbidities in this setting of cancer survivors.

C060

QUALITY OF LIFE DURING EARLY PHASE OF HEMOPOIETIC STEM CELL TRANSPLANTATION

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Introduction: Quality of life (QoL) evaluation during daily clinical practice among patients undergoing stem cell transplantation (SCT) assumes high relevance, due to the possibility to identify the heaviest complaints and to direct interventions to prevent and treat QoL deterioration. *Aim:* To analyze Quality of life (QoL) trend during the early post-transplant phase among patients transplanted in the six center of the Rome Transplant Network (RTN). *Methods:* EORTC QLQC30 questionnaire was selected as QoL evaluation instrument and administered at three time points: base line (t0), during aplasia (ta) and just before patient discharge (t1). Filled out questionnaires were sent to the centralized QoL evaluation unit to be processed; a patient report was returned containing: i. QoL scores and in-words description ii. suggestions concerning supportive care measure to improve QoL. Scores at t0, ta and t1 were compared (t-test) to search for variations over time (t0-ta and ta-t1). *Results:* From January to October 2016 in the RTN centers 163 SCT were performed; QoL questionnaires were administered during 83 SCT (51%); SCT with QoL assessments in each of the three time points (t0,ta,t1) were 42, corresponding to 42 patients; 23 males and 19 female; median age 56 (range 25-67) years; multiple myeloma 21, lymphoma 18, acute leukemia 3; autologous SCT 40 and allogeneic 2; disease status: complete remission or very good partial remission 32, other disease status 10. From t0 to ta, we observed a statistically significant deterioration of all QoL scales, except for three items: emotional scale, dyspnoea and financial issues; role scale resulted as the most deteriorated functional scale (-45.3% from t0 to ta), whereas gastrointestinal tract symptoms (nausea/vomiting, anorexia and diarrhea) were the most increased (>500% increase from t0 to ta). From ta to t1 we observed a statistically significant variation (improvement) of three items only: global QoL score, cognitive scale and pain scale (Table 1). Conclusion: SCT induced a marked deterioration of QoL in the early phase and few items recovered (partially) before discharge. Gastrointestinal tract symptoms were the most intensively influenced by SCT, so that measures of prophylaxis and treatment of QoL deterioration should be primarily directed towards them. QoL assessment and supportive care (motor rehabilitation psychological support, nutritional support, symptoms management) should be continued after discharge.

Table 1. Score and score changes of QoL items during early post-SCT phase.

	t0	ta-t0 %		ta	t1-ta %		t1
Global	68,5	-37,4	p<0,05	42,9	22,8	p<0,05	52,6
Physical	81,7	-25,6	p<0,05	60,8	11,8	NS	68,0
Role	74,6	-45,3	p<0,05	40,8	3,5	NS	42,3
Emotional	79,6	-6,5	NS	74,4	6,9	NS	79,6
Cognitive	86,5	-19,3	p<0,05	69,8	15,9	p<0,05	81,0
Social	77,8	-29,6	p<0,05	54,8	8,0	NS	59,1
Fatigue	26,5	111,0	p<0,05	55,8	-3,8	NS	53,7
Nausea/vomiting	4,0	1170,0	p<0,05	50,4	-25,2	NS	37,7
Pain	17,9	124,4	p<0,05	40,1	-37,6	p<0,05	25,0
Dyspnoea	15,9	45,0	NS	23,0	-34,5	NS	15,1
Insomnia	15,9	145,0	p<0,05	38,9	-6,1	NS	36,5
Anoressia	7,9	670,0	p<0,05	61,1	-6,5	NS	57,1
Stipsi	9,5	166,7	p<0,05	25,4	-37,5	NS	15,9
Diarrea	4,8	550,0	p<0,05	31,0	0,0	NS	31,0
Financial	15,9	-5,0	NS	15,1	-26,3	NS	11,1
QLQC30 summary score	84,5	-29,6	p<0,05	59,5	10,9	NS	65,9

Acute Leukemias 2

C061

NATIONAL EARLY WARNING SCORE (NEWS) AND QUICK SEQUENTIAL (SEPSIS-RELATED) ORGAN FAILURE ASSESSMENT (QSOFA) IN FEBRILE NEUTROPENIA OF ACUTE MYELOID LEUKEMIA PATIENTS

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Chemotherapy induced neutropenia in acute myeloid leukemia (AML) patients is at high risk for life threatening infections. Early diagnosis and prompt interventions are associated with better outcomes but is impeded by a lack of diagnostic tools. Recently the quick Sequential (Sepsis-related) Organ Failure Assessment (qSOFA) as well as the National Early Warning Score (NEWS) were proposed predicting in-hospital mortality. The aim of the present study was to assess the predictive validity of the NEWS and the qSOFA in a large cohort of homogeneous AML patients during neutropenia. A total of 1048 neutropenic episodes of 334 consecutive adult patients were analyzed. After induction chemotherapy a complete remission was achieved in 238 patients (71%), 74 were resistant (22%) and 22 died (7%). NEWS is a warning clinical score that evaluates seven clinical parameters. The qSOFA score consists of three parameters, each of which are allocated with one point: respiration rate > or =22/min, altered mental status and systolic blood pressure < or =100mmHg. Both scores were evaluated during a total of 2792 days of neutropenic fever. In addition clinical conditions such as septic shock, amine necessity, ventilation support, intensive care unit (ICU) admission and death were analyzed. The ability of the NEWS and the qSOFA score on prediction of early mortality was analyzed by calculating the area under the curve (AUROC) using the logistic regression model. Determination of the NEWS and qSOFA predicted statistically significantly ICU admission of the same day (NEWS AUROC 0.917, qSOFA AUROC 0.916) as well as death on the day of score determination (NEWS AUROC 0.984, qSOFA AUROC 0.969). Further, both scores were able to predict statistically significantly ICU admission after 24 hours (lag-1 analysis) (NEWS AUROC 0.929, qSOFA AUROC 0.913) and as well as death after 24 hours (NEWS AUROC 0.928, qSOFA AUROC 0.887). To our knowledge the present analysis is the first validation of the NEWS and the qSOFA during neutropenia in a large homogenous AML patients cohort. The NEWS and qSOFA may be valid tools to evaluate critical patients in intensive chemotherapy induced aplasia.

C062

INTERFERON GAMMA PRODUCTION BY ACUTE MYELOID LEUKEMIA CELLS UPREGULATE INDOLEAMINE 2,3-DIOXYGENASE (IDO) ENZYME IN LEUKEMIC MESENCHYMAL STROMAL CELLS

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Mesenchymal stromal cells (MSCs) substantially contribute to the creation of hemopoietic niche by regulating hematopoietic stem cell fate. Moreover, MSCs have a unique immune-modulating capacity. Although recent findings have outlined a putative MSC role in hematological malignancy development, MSC-dependent mechanisms potentially supporting leukemia remain unclear. The Indoleamine 2, 3-dioxygenase (IDO)1 pathway is a well-known nodal modifier of MSC immune-modulatory properties. Furthermore, IDO1 acts to induce immune tolerance in different settings, including acute myeloid leukemia (AML). We hypothesize that an intrinsic defect of leukemic MSCs or a reprogramming process induced in MSCs by AML cells may support leukemia. To this hypothesis: 1) we characterized MSCs isolated from AML patients (AML-MSCs) at diagnosis; 2) we studied IDO1 pathway as a putative tumor-supportive MSC-dependent mechanism. We efficiently isolated and expanded AML-MSCs. We first analyzed their phenotypic and functional properties compared to that of healthy donor-derived MSCs (HD-MSCs). We found that AML-MSCs express typical MSC markers and differentiation capacity. We also found that the frequency of rescued MSCs was lower in AML group than in HD, suggesting a reduced number of MSC precursors in leukemic bone marrow. AML-MSCs, analyzed by FISH, do not show AML cell cytogenetic abnormalities suggesting that AML-MSCs do not derive from the original malignant clone. However, AML-MSCs show a reduced proliferative capacity reflecting some intrinsic defect. Next, we investigated IDO1 expression and function. We demonstrated that IDO1 is expressed and efficiently up-regulated by inflammatory stimuli, in particular by Interferon(IFN)-gamma, in both HD-/AML-MSCs. Interestingly, we found that IDO1 is up-regulated in HD-/AML-MSCs as well as in AML cells after co-culture in the absence of exogenous stimuli. Among putative mechanisms able to induce IDO1 expression in MSCs we investigated IFN-gamma production and we found that AML cells expressed IFN-gamma before and after co-culture with MSCs. Moreover, an IFN- receptor neutralizing antibody reduced the up-regulation of IDO1 in MSCs co-cultured with AML cells. Our data suggest that IDO1 may be up-regulated in MSCs by a leukemic inflammatory microenvironment favoring an immune-tolerant and leukemia supporting milieu. Overall, our results would likely contribute to unravel signaling pathways underlying MSC–dependent mechanisms promoting leukemia.

C063

THE CHOICE OF CUT OFF VALUE AT DIFFERENTTIME POINTS IS CRUCIAL TO IMPROVE THE PROGNOSTIC ROLE OF MINIMAL RESIDUAL DISEASE EVALUATIONIN ACUTE MYELOID LEUKEMIA

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Despite the achievement of hematological remission (CR) acute myeloid leukemia (AML) patients with persistence of detectable disease assessed with high sensitivity techniques (multicolor-flow-cytometry, MFC, or PCR-based molecular analysis) show a poor outcome. A consensus on the most informative time-points (TPs) and cut-offs for minimal residual disease (MRD) assessment has not been reached. With the aim of evaluating the prognostic value of MRD assessment by identifying the most useful TPs, MFC and molecular positivity cut-off values, we retrospectively analyzed data of 110 consecutive AML patients treated in our center between 2004 and 2014. Median age was 47 years. All patients had received a fludarabine-containing induction. Three MRD TPs have been considered: TP1, after induction I; TP2, after induction II; TP3, after consolidation therapy for patients who did not undergo hematopoietic stem cells transplantation or just before transplant. MFC-MRD had been performed through 4-colour analysis (and 8-colour from 2013). Two cut-offs of MFC-MRD positivity were compared: a threshold of 2.5 10 4 residual leukemic cells (>0,025%) or 1 x 10-3 (>0.1%).

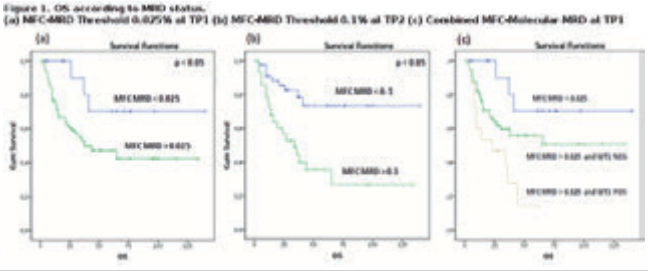

Figure 1.

For AML with mutated NPM1, molecular-MRD was evaluated and a reduction >3.5log of NPM1 transcript at TP1 was considered optimal as per our published experience. For patients with WT1 over-expression at diagnosis WT1-MRD was evaluated at TP1, considering WT1 negativity with a cut-off of WT1 copies/10^4 ABL lower than 250. CR rate after induction I and II was 82.7 and 85.5% and 2 years OS was 60.2% (median not reached). Multivariate Cox-Proportional Hazard model showed that MFC-MRD>0.1% at TP2 was the strongest predictor of

higher risk of death, whereas MFC-MRD <0.025 at TP1 was the stronges tpredictor of long survival (Figure 1, a-b). However, patients with MFC-MRD >0.025 at TP1 displayed a heterogeneous outcome. In this subgroup WT1-MRD at TP1 was able to identify patients with the higher risk of death (Figure 1c). Thirty-five patients carried NPM1-mutation. Two-years OS for NPM1-mut patients showing more or less than 3.5 log transcript reduction at TP1 was 94.1% *vs* 58.3%, respectively (p 0.039); 2 years OS for patients achieving NPM1-MRD negativity at TP2 was 90.5% *vs* 42.9% (p 0.003). Our data confirm the strong prognostic impact of MRD evaluation in AML treatment. Different MRD cut-offs at different TPs can give useful prognostic information that may drive post-induction therapy.

C064

ALTERATIONS IN NECROPTOSIS PATHWAY AFFECT PROGNOSIS OF PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Necroptosis is a type of necrotic cell death involving several genes transcription and activation of molecular mechanisms as death receptors, interferon, toll-like receptors, intracellular RNA and DNA sensors. The process is leading by the family of receptor-interacting protein kinase (RIPK3, RIPK2, RIPK1) and the MLKL substrate. Losses of RIPK3 or MLKL, as well as deficiency in apoptosis, could allow tumor cells to escape the immune-mediated cells death (ICD). We want to investigate the role of necroptosis deficiency in correlation with chemotherapy resistance and its impact as prognostic factor in AML. We performed SNP Arrays (Cytoscan HD and SNP 6.0, Affymetrix) on a cohort of 300 non-M3 AML patients at diagnosis and we analyzed the Overall Survival (OS) of our patients with deficiency on necroptosis pathways. Survival was analyzed with Kaplan-Mayer method and Log-Rank test. We further analyze the relevance of different prognostic factors by the use of COXHazard Ratio statistical analysis. We found that 18 patients presented a loss of RIPK1 or MLKL (nobody presented losses in RIPK3/RIPK2) and 13/18 patients were older than 65 years old. The Overall Survival (OS) of patients with alterations in these genes is significantly lower than control group, with a median OS of 3 *vs* 6 month respectively (p<.0.001). With Fisher Exact Test we further demonstrate that copy number loss of RIPK1 or MLKL are associate to loss of TP53 or FANCA genes, complex karyotype and advanced age. COX-Hazard Ratio model with RIMK1 or MLKL loss, BRCA1 loss, TP53 mutation, FANCA loss, secondary disease and diagnosis karyotype considered as categorical variable show that necroptosis deficiency (HR 1.98, CI 95% 1.04-3.78) TP53 mutation, and secondary AML are independent negative prognostic factors in an optimal model. Our study shows that losses in necroptosis pathways are an uncommon alteration in AML, prevalent in old population. Moreover, we hypothesize that the loss of genes involved in necroptosis could be a real mechanism of tumor immune-escape and could be a rational to select patients that high probability to be resistant at chemotherapy promoting ICD mechanism.

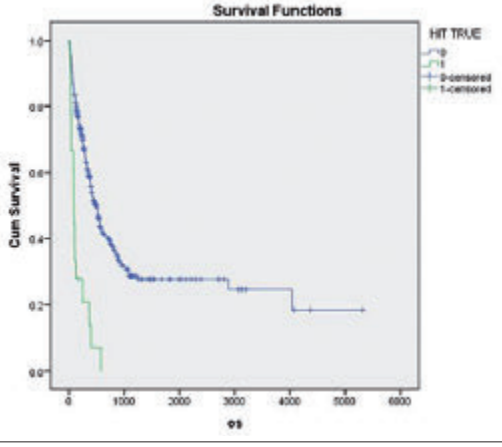

Figure 1.

C065

DISTINCT PATTERN OF ALTERATIONS IN TP53 MUTATED/DELETED AND WILD TYPE HIGH RISK ACUTE MYELOID LEUKEMIA (AML) PATIENTS: IDENTIFICATION OF NEW "TARGETTABLE" GENES/PATWAYS

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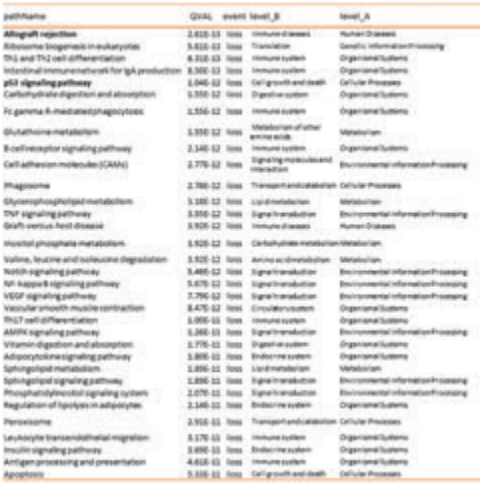
Background: The reported TP53 mutation rate in AML is relatively low (7.5-9%, TCGA) and predict a poor prognosis. In 2017 European LeukemiaNet recommended for AML to add TP53 mutations (muts) in the risk stratification. Specific chromosomal aneuploidies are closely correlated with each other and with presence of TP53 muts. *Aims:* Considering that TP53 mut AML pts have HRisk and no target therapy, we would identify genes/pathways that are mainly CNA-affected (Copy Number Alteration) in the mut TP53 group compared to the wt one. Patients and *Methods:* 358 adult AML pts were screened for TP53 muts. 219/358 samples were genotyped with SNP arrays. CNA analyses were performed using two software to confirm or integrate karyotype data. Fisher's exact test and pathway enrichment analyses were performed. *Results:* We detected TP53 muts in 51/358 (14.2%) pts. Mostly (34/51) of the TP53 mut pts (66.7%) had complex karyotype. TP53 alterations were significantly associated with poor outcome (OS and EFS p<0.0001). On TP53 locus, we matched CNA and cytogenetic analyses results. We identify 22 mutated pts that were also deleted (alt) and 7 pts that presented only a TP53 deletion. Therefore 43% of mut pts present a concomitant deletion. OS of TP53 alt pts is not statistically inferior respect to mut pts (p=0.77). Comparing 51 TP53 alt and 168 TP53 wt pts CNAs results that: a) chrs significantly altered are 5q, 17p, 12p; b) TP53 CNAs are present in the 44% of TP53 alt *vs* 0.63% of wt pts (Q=4.19E-11); c) over 11483 genes are differentially involved (mainly in Loss); d) that pathway categories mainly enriched are Immune System, Metabolism, Signal Transduction (Table 1); e) TP53 deletion seems less deleterious (in terms of OS) than TP53 mutation or TP53 alt; f) "p53 signaling pathway" is one of the most affected in TP53 alt pts (Q=1.04E-12).

Table 1. High significative pathway enrichment (KEGG database) of TP53 altered AML pts compared to wt.



Conclusions: TP53 muts with or without deletion were predicted to be deleterious and significantly correlated with worse prognosis. For these reasons, TP53 mutation/deletion screening should be recommended. Different pattern of alterations in alt and wt groups have to be deeper investigated to discover targetable nodes of this complex network. Plans: we are going to: a) test *in vitro* drug/s that have as target pivotal gene/s; b) identify pathway enrichment in the 3 distinct AML groups (TP53 mut; TP53 mut/del; wt); c) as point a) but considering the 3 groups. ELN, AIL, AIRC, PRIN, Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL; HARMONY.

Non Hodgkin Lymphomas 2

C066

HIGH RISK AGGRESSIVE B-CELL LYMPHOMAS IDENTIFIED BY FISH: A MULTICENTRIC ITALIAN RETROSPECTIVE STUDY

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Double-hit lymphomas (DHL) are high-risk, non-Hodgkin B-cell lymphomas with dismal prognosis. The definition of DHL based on MYC, BCL2 and BCL6 abnormalities by FISH analysis is debated, as well as the optimal treatment strategy. This multicentric, retrospective study aimed to characterize the clinical landscape of DHL in Italy, describing the current diagnostic paradigms and the preferred therapeutic choices. Patients (pts) with newly diagnosed diffuse large B-cell lymphoma (DLBCL) or "unclassifiable" aggressive B-cell lymphoma (BCLU) with at least one FISH alteration on MYC, BCL2 or BCL6 loci were included. The main endpoints were clinical response rate, progression-free survival (PFS) and overall survival (OS). Data were analyzed by Stata 13 software. A total of 95 pts with DLBCL (2010-2016) were firstly screened [33 BCLU and 62 DLBCL NOS] among 17 Italian Institutions belonging to the Fondazione Italiana Linfomi. The MYC FISH was abnormal in 80/95 pts, accounting for 72 translocations. Of these pts, 52 (72%) were further studied for BCL2 and 41 (60%) for BCL6. Out of the 72 MYC translocated pts, 35/72 (49%) were classified as DHL [21/35 (60%) MYC/BCL2, 14/35 (40%) MYC/BCL6] and 3/72 (4%) as "triple hit" lymphoma [(THL) - MYC/BCL2/BCL6].

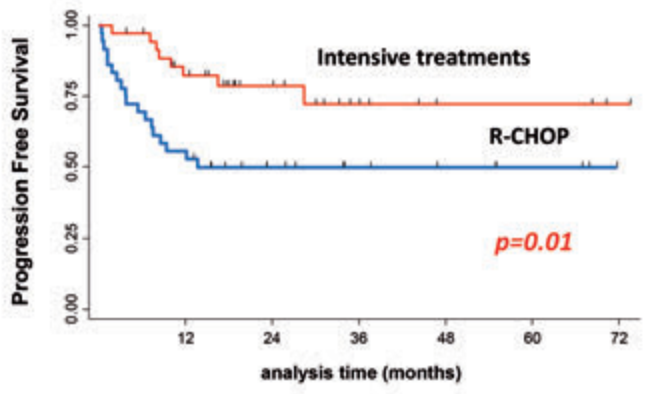

Figure 1. PFS in 72 pts according to received treatment.

Median age was 62 years, (19-84), 42 males. Thirty-one (43%) showed ECOG-PS>2, 50 (69%) stage >2, 31 (43%) >1 extranodal site, 51 (71%) elevated LDH and 40 (56%) bulky disease (>6 cm). Therefore, 36 pts (50%) had intermediate and 24 (33%) had high IPI risk group. Fifty pts (69%) were defined as GCB according to the Hans algorithm. The most common upfront regimen was R-CHOP (n=36), followed by intensive regimens (R-hyperCVAD/MA or BFM, n=26) and DA-EPOCH-R (n=10). The median follow up of survivors was 23 months. The CR rate was 67% in the entire cohort and 61% in DHL and THL. The projected 2 years PFS and OS of the overall population were 64% (95% CI 51-74) and 70% (95% CI 57-80), respectively. According to received treatment, the 2 years PFS was 80% (95% CI 60-64) in pts who received intensive treatments and 50% (95% CI 33-65) in pts who received R-CHOP (p=0.01), independently from the age of the pts (Figure 1). This is the first multicenter, retrospective study collecting DHL cases in Italy. Although a fully annotated FISH profile is not always available in all centers, our preliminary data suggest that intensified treatments might induce a PFS advantage. An extension of the series is needed to strengthen the data.

C067

INTERFERON-FREE ANTIVIRAL TREATMENT IN B-CELL LYMPHOPROLIFERATIVE DISORDERS ASSOCIATED WITH CHRONIC HEPATITIS-C VIRUS INFECTION

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Introduction: Regression of hepatitis C virus (HCV)-associated lymphoproliferative disorders with interferon(IFN)-based antiviral treatment was the strongest evidence for an etiological link between lymphoma and HCV infection (NEJM 2002). To confirm this hypothesis we have recently reported data on IFN-free regimens combining direct-acting antivirals (DAAs) therapy in indolent HCV-positive B-cell non-Hodgkin lymphoma (NHL) (Blood 2016). *Methods:* We analyzed virological and lymphoproliferative disease response (LDR) of 97 patients with indolent B-cell non-Hodgkin lymphomas (NHL) or chronic lymphocytic leukemia (CLL) and chronic HCV infection treated with DAAs and their outcome. *Results:* Histological distribution was as follows: 69 marginal zone lymphomas (MZL), 6 lymphoplasmacytic lymphomas, 8 follicular lymphomas, 10 CLL/small lymphocytic lymphoma (SLL), 4 low-grade NHL non otherwise specified. All but thirteen patients received a Sofosbuvir-based regimen. Median duration of DAA therapy was 12 weeks (range 4-24 weeks). A sustained virological response at week 12 after finishing DAAs (regarded as cure of HCV) was

obtained in 99% of patients; overall LDR rate was 73% including 29 patients (30%) achieving a complete response and 41 (43%), a partial response. After a median follow up of 15 months, 2-year progression-free and overall survival were 70% [95% confidence interval: 49% - 83%] and 97% [88-99%], respectively. *Conclusions:* DAA therapy induces a high LDR rate in HCV-associated lymphoproliferative disorders and confirms the role of HCV in lymphomagenesis. This chemotherapy-free approach should be considered first-line therapy in patients without need of urgent treatment. Prospective trials are eagerly awaited in this setting.

C068

EVALUATION OF TENASCIN-C BY TENATUMOMAB IN T-CELL NON-HODGKIN LYMPHOMAS IDENTIFIES A NEW TARGET FOR RADIOIMMUNOTHERAPY

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Background: With currently available treatments, the prognosis of T-cell non-Hodgkin lymphoma (NHL) is poor and the identification of new targets is crucial to develop innovative therapeutic strategies. Tenascin-C, a large extracellular glycoprotein, can be recognized by the monoclonal antibody Tenatumomab. Under physiological conditions tenascin-C is not expressed in adult life, but it is overexpressed in cancer where it was associated with relevant neoplastic processes as angiogenesis. Aim of the study was for the first time to evaluate the presence and the clinical relevance of tenascin-C in T-cell NHL. *Methods:* We used a Tenatumomab-based immunohistochemistry approach to investigate the expression of tenascin-C in 75 systemic T-cell NHL, including 72 mature peripheral T-cell lymphoma (PTCL) and 3 precursor T-cell NHL, and 25 cutaneous T-cell lymphoma (CTCL) patients. Data were analyzed in terms of staining intensity, proportion of involved areas and histological pattern, and results were correlated with the baseline clinical characteristics and outcome. *Results:* Nearly all of the cases (93%) were tenascin-C positive and 59% of systemic T-cell NHL were characterized by the predominant positivity (>50% of involved areas). A stromal expression was present in all cases, while a vascular and vascular plus cytoplasmic positivity in 49% and 23%, respectively. In multivariate analysis, presence of vascular pattern of expression was associated to a significantly shorter overall survival. The constant overexpression of the tenascin-C gene was observed in two independent publicly available gene expression datasets of PTCL cases. *Conclusions:* Tenascin-C represents an attractive target that sets the rationale to investigate the therapeutic activity of radiolabeled Tenatumomab in T-cell NHL.

C069

MINIMAL RESIDUAL DISEASE (MRD) EVALUATION IN LYMPHOMAS WITHIN THE FIL (FONDAZIONE ITALIANA LINFOMI) MRD NETWORK: INTER-LABORATORY REPRODUCIBILITY ON BORDERLINE SAMPLES

I. Della Starza[1], M. Cavalli[1], L.A. De Novi[1], E. Genuardi[2], B. Mantoan[2], D. Drandi[2], L. Monitillo[2], E. Ciabatti[3], S. Grassi[4], A. Gazzola[5], C. Mannu[5], C. Agostinelli[5], P.P. Piccaluga[5], S. Galimberti[3], A. Guarini[1,6], R. Foà[1], M. Ladetto[7], S. Ferrero[2], I. Del Giudice[1]

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In 2009, the 4 laboratories of the Fondazione Italiana Linfomi (FIL)-minimal residual disease (MRD)-network started a collaborative effort to harmonize and standardize their methodologies, performing QC (Quality Control) rounds for follicular lymphoma (FL) and mantle cell lymphoma (MCL) MRD assessment. Pooling the molecular results of bone marrow (BM) samples analysis performed during the QC rounds, we aimed at determining how "borderline" (brd) samples (*i.e.* those with a low MRD level) challenge the inter-lab reproducibility and data interpretation. In 14 QC rounds between 2010 and 2016, the Network labs received 188 BM (114 FL and 74 MCL) samples; 167 were analyzed by both nested polymerase chain reaction (PCR) and real-time quantitative PCR (RQ-PCR) for BCL2/IGH MBR or IGHV rearrangements (Gribben, 1993; Ladetto, 2000; Van Dongen, 2003; Donoval, 2000). All analyses were conducted according to the EuroMRD Consortium guidelines (van der Velden, 2007). The labs reached a uniform sensitivity of 10(-5) and a quantitative range for RQ-PCR of at least 10(-4). Overall, 83% (139/167) BM samples were concordantly classified as PCR+/RQ-PCR+ or PCR-/RQ-PCR- by all the 4 labs. The remaining 28 (17%) samples resulted alternatively positive and negative in the inter-lab evaluations, representing brd samples: 8 were PCR-brd/RQ-positive not quantifiable (PNQ), 7 PCR-/RQ-brd, 2 PCR-brd/RQ- and 11 PCR-brd/RQ-PCR-brd. Thus, while in 17 cases the "brd status" was defined alternatively by only one method, 11/167 samples (6.6%) resulted brd by both techniques (Figure). Although all samples were tested in 3 replicates across the 4 labs, for a total of 12 replicates/sample, 31 brd samples were identified, 13 of which by both approaches: of 156 evaluations performed on the 13 brd, 69 (44%) were PCR-positive and 87 (56%) PCR-negative, 58 (37%) were RQ-PNQ and 98 (63%) RQ-negative. Despite the high inter-lab reproducibility in the MRD analysis obtained and maintained by the QC round strategy, samples with the lowest MRD levels can still represent a challenge: 17% of our samples showed discordant results in inter-lab assessments; 39% of them (11/28) remained brd even applying both methods. Results did not change even increasing the number of replicates/sample. Thus, although representing a minority, brd samples are still problematic, especially when a clinically oriented interpretation is required. In this context, alternative, novel methods such as digital PCR and NGS need to be tested.

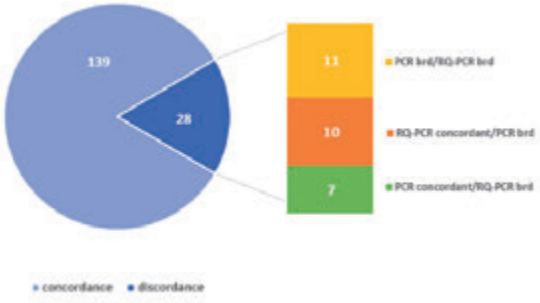

Figure 1. 167 BM samples analyzed by PCR and RQ-PCR. 139/167 samples were classified as positive or negative by all the FIL laboratories with both methods, while 28/167 showed discordant results in the inter-lab assessments. Among the 28 borderline (brd) samples, 21 resulted PCR brd and 18 RQ-PCR brd. In particular, 10 were concordant for RQ-PCR analysis and brd for PCR, 7 resulted concordant for PCR analysis and brd for RQ-PCR, 11 were classified as brd samples by both techniques.

C070

THE PROGNOSTIC ROLE OF CELL OF ORIGIN PROFILE, MYC, BCL2 AND TP53 IN UNTREATED POOR-RISK YOUNG DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: RESULTS OF FIL-BIODLCL04 STUDY

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On behalf of Fondazione Italiana Linfomi FIL, Hematology, Città della Salute e della Scienza Hospital and University

The phase III randomized study FIL-DLCL04 demonstrated that the intensification with Rituximab-MAD+BEAM and ASCT after an abbreviated Rituximab-dose-dense chemotherapy (R-HDC+ASCT) in young

poor-risk patients with untreated DLBCL, determined the superiority in term of failure-free survival (FFS) compared to a full course of R-dose-dense chemotherapy alone (R-dose-dense). However, this statically improvement in FFS did not translate in any overall survival (OS) advantage. The aim of BIO-DLCL04 was to correlate the cell of origin (COO) profile assessed by immunohistochemistry (IHC) and by nanostring and the presence of biomarkers (MYC, BCL2, TP53 Wild Type, Mutated) in IHC or fluorescent *in situ* hybridization (FISH), with OS and FFS. From 2005 to 2010, 399 DLBCL were enrolled in FIL-DLCL04 and randomized to receive R-HDC+ASCT in 199 and R-dose-dense in 200 (NCT00499018). Central histology revision was mandatory. Cases were classified for COO in germinal center (GC) and non-GC according to Hans' algorithm by IHC and in GB, activated B-cell (ABC) and unclassified (UNK) by Nanostring. BCL2, MYC and TP53 anomalies were tested by FISH. OS and FFS were analyzed; a crude hazard ratio (HR) and an adjusted HR (aHR) for age, treatment, gender, age-adjusted International Prognostic Index, performance status, bone marrow involvement were calculated. Ninety-five DLBCL were analyzed for all the planned analyses. No selection bias was observed between the 95 cases and the global FIL-DLCL04 study populations. Only 5 cases were defined as double-hit; due to the small numbers, a separate analysis was not performed. At a median follow-up of 72 months, 5-years OS and FFS for COO by IHC and Nanostring, MYC and BCL2 by FISH and TP53 were reported in table 1. No significant differences by R-HDC+ASCT *versus* R-dose-dense were observed in the different biological subgroups (data not shown). In conclusions, in our prospective FIL-BIODLCL04 trial, COO assessed by IHC is not predictive of outcome; Nanostring is able to discriminate two groups at different prognosis, GC and ABC. In our series, with the limit of small numbers, MYC and BCL2 did not affect OS and FFS. An important role is played by TP53, that represent a factor that impact on OS and FFS. The intensification with R-HDC+ASCT is not able to overcome the dismal prognosis of the unfavorable subgroups.

Table 1. 5-years OS and FFS for COO by IHC and Nanostring, MYC and BCL2 by FISH and TP53.

	N of pts	5-year OS, % (95% CI)	HR (95% CI)	aHR (95% CI)	5-year FFS, % (95% CI)	HR (95% CI)	aHR (95% CI)
GC IHC	37	76 (58-86)	1	1	59% (42-73)	1	1
nonGC IHC	57	77 (63-86)	0.93 (0.4-2.18), p.87	0.89 (0.35-2.26), p.813	70% (56-80)	0.64 (0.32-1.28), p.208	0.51 (0.24-1.09), p.081
GC Nano	55	85 (73-92)	1	1	69 (55-80)	1	1
ABC Nano	25	56 (35-73)	3.72 (1.49-9.29), p.005	4.59 (1.55-13.59), p.006	52 (31-69)	1.55 (0.74-3.24), p.249	1.3 (0.56-3.03), p.0.539
UN Nano	15	73 (36-91)	1.34 (0.35-5.03), p.0669	0.88 (0.21-3.58), p.855	77 (43-92)	0.54 (0.16-1.85), p.33	0.33 (0.09-1.22), P.096
MYC-	80	78% (67-86)	1	1	69% (57-78)	1	1
MYC+	8	63% (23-86)	1.75 (0.51-5.99), p.37	1.65 (0.35-7.66), p.524	50% (15-77)	1.8 (0.63-5.19), p.273	2.01 (0.6-6.77), p.258
BCL2-	73	79% (68-87)	1	1	70% (58-79)	1	1
BCL2+	19	68% (43-84)	1.72 (0.67-4.44), p.262	1.65 (0.35-7.66), p.524	53% (29-72)	1.88 (0.87-4.09), p.11	1.48 (0.66-3.32), p.341
TP53 WT	8	81% (70-89)	1	1	73% (61-82)	1	1
TP53 Mut	70	33% (6-66)	4.33 (1.54-12.21), p.006	3.16 (0.81-12.4), p.099	19% (1-53)	3.78 (1.5-9.49), p.005	2.33 (0.77-7.08), p.136

Myeloma and Monoclonal Gammopathies 2

C071

CARFILZOMIB, BENDAMUSTINE AND DEXAMETHASONE (CBD) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA: RESULTS OF A PHASE I/II EMN STUDY

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In patients with relapsed/refractory multiple myeloma (rrMM) the combination of bendamustine, a bifunctional alkylating agent, with bortezomib and a corticosteroid has shown activity and safety profile in several phase II studies. Moreover, triplet regimen including carfilzomib, a second generation proteasome inhibitor with limited neurotoxicity, cyclophosphamide and dexamethasone has been reported to be well tolerated and active in the frontline setting. Based on these data we evaluated the combination carfilzomib, bendamustine and dexamethasone (CBd) in rrMM. The primary objective of the phase I part of the trial was to determine the maximum tolerated dose (MTD) of CBd combination whereas that of the phase II was to determine the rate of very good partial remission (VGPR). Patients with rrMM with at least two lines of prior therapy received a 4 week cycle of bendamustine 70 mg/mq on day 1 and 8; carfilzomib at escalating dose from 27 mg/mq to 45 mg/mq on day 1, 2, 8, 9, 15, 16 (20 mg/mq on days 1, 2 in the first cycle); dexamethasone 20 mg/ mq on day 1, 2, 8, 9, 15, 16, 22 and 23. After 8 cycles, responding patients received maintenance therapy with carfilzomib plus dexamethasone on two consecutive days every 14 days until progression. After the first six patients were entered at the starting dose of 27 mg/mq, carfilzomib dose was escalated to 36 mg/mq but two patients developed pneumonia so the recommended phase II dose was 27 mg/mq. As of April 2017, enrollment was completed at 63 patients. Currently 41 patients are evaluable. Median age was 67 years, 61% had ECOG PS 1, 66% R-ISS II/III and median time from diagnosis was 5.8 years. Patients received a median of 4 (2-6) previous lines of therapy, 88% received prior bortezomib, 88% prior immunomodulatory agents and 85% autologous stem cell transplant. Moreover, 34% of patients had a relapse and refractory disease. The most common study treatment grade 3 adverse events were neutropenia (27%), thrombocytopenia (24%), infection (12%), particularly pneumonia. Three patients (7%) developed cardiac and three (7%) thromboembolic events. After a median follow-up of 5.95 months, median progression free survival was 11.4 months and 1-yr overall survival 75%. In conclusion, CBd combination demonstrated promising activity in old heavily pretreated MM patients although anti-infective prophylaxis should be mandatory and cardiovascular functions strictly monitored. Results will be update at the meeting.

C072

PROGNOSTIC VALUE OF ADDITIONAL CHROMOSOMAL COPY NUMBER ALTERATIONS IN EITHER HYPERDYPLOID OR NON-HYPERDIPLOID NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS ENROLLED IN THE EMN02 STUDY

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Patients with Multiple Myeloma (MM) can be hierarchically subdivided into hyperdiploid (H) and non-hyperdiploid (NH) subgroups, according to the primary genetic events involved in the pathogenesis of the disease. Secondary genetic "hits" may contribute to a selective advantage and ultimately lead to tumour progression. Copy Number Alterations (CNAs) are frequent chromosomal events in MM and can help to further refine the prognosis of this genetically heterogeneous disease. Aim of this study was to evaluate whether additional CNAs, when present either in an H or in a NH genomic background, might differentially affect the outcomes of newly diagnosed MM patients who were treated with either standard-dose intensification therapy (VMP) or high-dose intensification melphalan followed by either single or double autologous stem cell transplantation in the context of the EMN02 phase III trial. Genomic data were obtained from 256 patients, whose highly purified CD138+ BM plasma cells were profiled both by FISH and SNPs array. Providing that an underlying different biology supports the stratification of MM according to the presence of 3N odd chromosomes, patients included in the study were divided into H (n=137) and NH (n=117) subgroups. Patients in both subgroups were homogeneous with respect to clinical characteristics at baseline, whereas an over-representation of both structural and CN aberrations was found to characterize the genomic landscape of NH patients. 51/137 (38%) H patients and 44/117 (38%) NH patients relapsed or progressed after a median of 45 and 44 months. In a univariate analysis, several additional structural and CN aberrations, which are listed in Table 1, were found to differentially affect the clinical outcomes of H and NH patients. Overall, the prognosis of NH patients was adversely affected by the presence of TP53 losses and CKS1B gains, and by randomization to receive VMP intensification therapy. On the contrary, H patients who carried either IgH translocations (an event rarely observed) or deletions affecting chromosomes 1p (FAM46C), or 17p (TP53), or 12p, or 8p, or 13q (RB1), or who were randomized to VPM had a worse outcome, as compared to the others. These results further corroborate the hypothesis that additional CNAs can differently affect the outcomes of MM patients, thus helping to more accurately identify those who are at higher risk.

Table 1.

Table 1. progression rates, TTP, PFS and OS were evaluated in pts stratified according to the listed factors; table reports p values of the exact test for progression rates comparison and for the univariate analysis of TTP, PFS and OS.

	progression rates		TTP		PFS		OS	
	H	NH	H	NH	H	NH	H	NH
randomization	0.03	ns	0.02	0.007	0.01	0.007	ns	0.04
RB1 CN loss	0.05	ns	0.07	ns	0.07	ns	0.01	ns
FAM46C CN loss	ns	0.04	0.03	ns	0.03	ns	<0.001	ns
chr12p del	ns	ns	ns	ns	ns	ns	0.001	ns
chr8p del	ns	0.02	ns	ns	ns	ns	0.03	ns
chr12p and/or chr8p del	ns	ns	0.07	0.09	0.07	0.09	<0.001	0.001
TP53 CN loss	ns	0.004	0.03	0.008	ns	0.07	0.04	ns
CKS1B CN gain	ns	0.001	ns	0.03	ns	ns	ns	0.09
IgH translocation	0.002	ns	ns	ns	0.03	ns	ns	ns

CN = Copy Number; chr = chromosome; TTP = Time To Progression; PFS = Progression Free Survival; OS = Overal Survival

C073

MINIMAL RESIDUAL DISEASE BY MULTIPARAMETER FLOW CYTOMETRY IN TRANSPLANT ELIGIBLE PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA ENROLLED IN THE EMN02/HO95 PHASE 3 TRIAL

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Background: Minimal residual disease (MRD) detection is a sensitive strategy to measure response in multiple myeloma (MM). We assessed MRD by multiparameter flow cytometry(MFC) in patients with newly diagnosed MM enrolled in the EMN02/HO95 phase 3 trial. *Methods:* Patients aged ≤65 years were treated with Bortezomib-Cyclophosphamide-Dexamethasone (VCD) induction, mobilization and stem cell collection, intensification with Bortezomib-Melphalan-Prednisone (VMP) *vs* High-Dose-Melphalan (HDM) followed by stem cell transplant, consolidation with Bortezomib-Lenalidomide-Dexamethasone (VRD) *vs* no consolidation, and Lenalidomide maintenance. MRD was assessed in patients achieving at least a very good partial response (VGPR) before maintenance (after HDM, VMP or VRD) and during maintenance every 6-12 months; samples were centralized to 3 European labs. MFC was performed on bone marrow according to Euroflow-based methods (8 colors, 2 tubes) with a sensitivity of 10-5. Quality checks were done to compare sensitivity and to show correlation between protocols (Hofste op Bruinink D, ASH 2016 abstract 2072). *Results:* 316 could be evaluated before maintenance: median age was 57 years, 18% (57/316) had ISS III and 22% (70/316) had high risk cytogenetic abnormalities [either one among del17, t(4;14) or t(14;16)]; 63% (199/316) had received HDM and 37% (117/316) VMP; thereafter 51% (160/316) had received VRD. After a median follow-up of 30 months from MRD enrolment, 76% (239/316) patients were MRD-negative: 64% (153/239) in the HDM *vs* 36% (86/239) in the VMP groups. 3-year PFS was 50% in MRD-positive *vs* 77% in MRD-negative patients (HR 2.87, 95% CI: 1.75 - 4.72; p<0.001). By subgroup analyses according to baseline features and therapies, high risk cytogenetic abnormalities were the most important risk factors for MRD-positivity (HR 9.87, 95% CI: 4.3 – 22.63; interaction-p=0.001). 48% of MRD-positive patients at pre-maintenance who had a second MRD evaluation after ≥1 year of lenalidomide became MRD-negative. *Conclusions:* MRD by MFC is a strong prognostic factor in MM patients receiving intensification with novel agents or transplant; lenalidomide maintenance further improved depth of response; high risk cytogenetic abnormalities are the most important prognostic factors in MRD-positive patients.

C074

18F-FDG PET/CT FOR THE EVALUATION OF METABOLIC RESPONSE TO THERAPY IN NEWLY DIAGNOSED TRANSPLANT ELIGIBLE MULTIPLE MYELOMA (MM) PATIENTS: RESULTS FROM THE IMAGING SUB-STUDY OF THE EMN02/HO95 MM PROSPECTIVE RANDOMIZED PHASE III TRIAL

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FDG-PET/CT is nowadays the preferred imaging technique to assess and monitor the metabolic response to therapy. One of the major limitation of PET/CT is the lack of standardization in interpreting the results. Aim of the present study was to prospectively evaluate FDG-PET/CT at diagnosis, after 4 cycles of induction therapy and prior to maintenance in a sub-group of patients enrolled into EMN02 phase III trial for patients with newly diagnosed transplant eligible symptomatic MM, as previously reported. In particular, the two primary endpoints were firstly to assess the value of PET/CT as a tool to evaluate metabolic response to therapy and secondly to standardize PET/CT evaluation by centralized imaging and revision and definition of prognostic cut-offs. 103/718 patients enrolled in the trial from 02/2011 through 04/2014 were included in the PET/CT sub-study, and followed for a median of 24 months. PET/CT was performed in each of the 8

participating centres and was a posteriori re-interpreted in a blinded independent central review process. The following characteristics were reported and scaled, according to Deauville criteria (score 1-5): bone marrow metabolic state (BM), number (Fx) and score (Fs) of focal PET positive lesions, presence and site of extramedullary disease (EM), SUVmax of the hottest lesion. Concordance among reviewers was highest for Deauville score 4 for all the parameters (Krippendorf's alpha coefficient >0.5). PET/CT was positive in 78% of the patients at baseline, with a median SUVmax of 6.0, in 59% after induction (SUVmax 3.7) and in 34% prior to maintenance (SUVmax 3.4). Normal PET/CT findings before maintenance were associated with a significant improvement in PFS and OS; in particular the most prognostic parameters were the presence of FLs, with a Deauville score ≥4 (P=0.001 for both PFS and OS) and Fx ≥ 4 (P=0.01 for PFS and 0.0002 for OS). Moreover, the negativization of Fs prior to maintenance as compared to baseline was associated with better PFS and OS (P=0.009 and 0.027, respectively). On the contrary, neither of the tested scores was prognostic for BM. The prognostic relevance of pre-maintenance PET/CT was retained across the randomization arm (VMP or ASCT). In conclusion, normalization of PET/CT before maintenance was associated with a significant improvement for PFS and OS. Deauville score 4 was identified as the most prognostic. BM metabolic state is not a good prognostic parameter to be tested by PET/CT.

C075

AN ITALIAN SINGLE CENTER PROSPECTIVE STUDY ON OUTCOMES IN AL AMYLOIDOSIS

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Background: In the last decade, new treatment strategies and patients selection according to biomarkers have modified the approach to patients with light-chain (AL) amyloidosis. However, prospective studies are hampered by patient selection, short follow-up and small numbers due to the rarity of the disease and the difficulties in enrollment. *Methods:* Starting in 1994, we protocolized the collection of parameters of clonal and organ disease at baseline and during treatment in all the 1933 patients diagnosed at our center. Here, we report the outcome of 1065 subjects enrolled after 2004, when systematic collection of cardiac biomarkers and FLC data started. *Results:* Median age was 65 years. Involved organs were heart (77%), kidney (66%), soft tissue (17%), liver (14%) and PNS (12%), >2 organs were involved in 24% of cases. Mayo stage was I in 17% of patients, II in 44% and IIIa (NT-proBNP cutoff <8500 ng/L) in 20% and IIIb (NT-proBNP cutoff >8500 ng/L) in 19%. Renal stage was I in 47% of patients, II in 40% and III in 13%. The median bone marrow plasma cells infiltrate was 11% (IQR: 7-25%). Most common upfront treatments were melphalan-dexamethasone [MDex, 367 patients, overall hematologic response (HR) 51%, very good partial or complete response (VGPR/CR) 38%], cyclophosphamide-bortezomib-dexamethasone [297 patients, HR 55%, VGPR/PR 44%), and bortezomib-MDex [132 patients, HR 64% (P=0.002 compared to MDex), VGPR/PR 50%]. Median follow-up of living patients was 42 months (IQR: 23-73 months). Fifty-six percent of patients died (13% within 3 months). Median survival in cardiac Mayo Stage I was not reached, in stage II 58 months, in stage IIIa 17 months and in stage IIIb 6 months (P<0.001). No overall survival advantage was noted in patients treated with upfront bortezomib based combinations compared to MDex. *Conclusion:* The outcome of patients with AL amyloidosis is extremely heterogeneous and a significant proportion of patients die before having the chance to benefit from treatment. The extensive use of upfront bortezomib ameliorates the quality of response, but does not translate in a general improvement of overall survival.

Anemias and Myelodysplastic Syndromes

C076

IMPACT OF GENE MUTATIONS ON RESPONSE TO LENALIDOMIDE AND OS IN LOWER-RISK NON-DEL(5Q) MDS PATIENTS INELIGIBLE/REFRACTORY TO ERYTHROPOIESIS-STIMULATING AGENTS (ESAS)

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We investigated the relationship between gene mutations, response and overall survival (OS) in the phase 3 randomized MDS 005 study of RBC transfusion-dependent (TD) lower-risk non-del(5q) MDS patients treated with lenalidomide.Eligible patients were RBC-TD (≥2 units RBCs/28 days) with IPSS-defined Low/Int-1-risk non-del(5q) MDS and ineligible/refractory to ESAs.239 patients were randomized 2:1 to lenalidomide 10 mg/day or placebo.DNA was isolated from BMMC or whole blood at screening and next-generation sequencing of 56 genes was performed.Fisher exact test was used to test mutation status association with response. Median OS was calculated by Kaplan–Meier and log-rank used to test treatment effect.Somatic mutations were evaluated in 198/239 patients (lenalidomide n=130,placebo n=68) and detected in 30/56(54%) genes and 173/198(87%) patients. The most frequently mutated genes were SF3B1 (59%), TET2(33%), ASXL1(23%), DNMT3A(14%); the most frequent comutations were SF3B1/TET2(23%), SF3B1/DNMT3A(10%), SF3B1/ASXL1(10%) and TET2/ASXL1(9%). 115/116 patients with SF3B1 mutations had ≥5% ring sideroblasts.The 56-day RBC transfusion-independence (RBC-TI) response rate was significantly lower in lenalidomide-treated ASXL1mutant *versus* wildtype patients (10.3%*vs*31.7%).9/16 lenalidomide-treated DNMT3Amutant patients achieved IWG-defined erythroid response, without correlation with comutations or DNMT3A mutation type;7/16 also achieved ≥8-week RBC-TI response,higher than wildtype patients although not significant(43.8%*vs*24.6%).RBC-TI response rate with lenalidomide was similar regardless of total number of mutations (range 0–5) per patient.Twelve lenalidomide-treated patients achieved RBC-TI ≥1 year;7/12 carried an SF3B1 mutation, either alone (n=3) or in combination with others (n=4). Higher numbers of mutations were significantly associated with worse median OS (P=0.0005). Mutation in any of the genes associated with a negative prognosis was significantly associated with worse median OS, irrespective of treatment (P=0.0003).Lenalidomide-treated DNMT3A-mutated patients had a trend for longer OS *versus* placebo.In this study,mutations in genes recurrently mutated in myeloid cancers were detected in 87% of patients.SF3B1 mutations were most frequent and not associated with response to lenalidomide. ASXL1-mutant patients had a significantly lower response rate *versus* wildtype patients,whereas DNMT3A-mutant patients had a trend for improved response and OS after treatment.

C077

FIRST LINE TREATMENT OF APLASTIC ANEMIA WITH THYMOGLOBULINE IN EUROPE AND ASIA: OUTCOME OF 955 PATIENTS TREATED 2001-2012

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Background: Recent studies have suggested inferior outcome of patients treated with rabbit ATG (Thymoglobulin) as compared to horse ATG (ATGAM, Pfizer or Lymphoglobulin, Genzyme- the latter no longer available); other studies have shown comparable responses and survival. However these studies are based on a relatively small number of patients and a short follow up. *Aims:* The aim of this study was to assess real life outcome of a large number of AA patients, treated in Europe and Asia with rabbit ATG (Thymoglobulin, SANOFI) and cyclosporin, as first line treatment. Material: Eligible for this study were patients with AA, treated with Thymoglobulin between 2001 and 2008 (n=492) and 2009-2012 (n=463) in Europe (n=498) or Asia (n=457). Median year of treatment, was 2008: characteristics were comparable : median age 20 and 21 years, interval diagnosis treatment (23 and 25 days) and severity of the disease (46% and 48% with vSAA) Results Early mortality. Mortality <90 days was significantly reduced from 5,5% and 2.1%, respectively, in the time period 2001-2008 and 2009-2012 (p=0.007). Response Cumulative incidence of response at 6 months, recorded in 779 patients, was age dependent: 68%, 66%, 62%, 40% respectively in patients aged 0-20, 21-40, 41-60, >60 (p=0.0006). The actuarial 10 year survival for the entire population was 71%, and 70%, when pts were censored as surviving at transplant. Actuarial 10 year survival in univariate analysis was as follows: 89% *vs* 61% for day 90 responders *vs* non responders (p<0.01), 68% *vs* 80% for males *versus* females (p=0.07); 82%, 72%, 66%, 27% in pts aged 0-20, 21-40, 41-60, >60 years (p<0.001); 67%, 78%, 76% in pts with neutrophils <0.2x10^9/L, 02-05x10^9/L and >0.5x10^9/L (p<0.001); 77%, 75%, 68% for pts with an interval diagnosis-treatment of <30 days, 31-60 days or > 60 days (p=0.002). Finally pts treated >2008 had a 5 year survival superior to pts treated before 2008 (84% *vs* 77%, p=0.01). Cox: In a multivariate Cox model negative predictive factors were increasing AGE, longer interval Diagnosis Treatment, year of treatment <2008, increasing severity Conclusions. With a current mortality (<day 90) of 1.4%, a response rate at 6 months of 60% and 5 year survival of 83%, the combination of CsA and Thymoglobulin appears to be safe and effective as first line treatment in patients aged 1-60. In patients over 60 years of age, early mortality is higher (9%), response rate (40%) and 5 year survival (67%) are lower. For patients treated within 1 month from diagnosis, current overall survival is 88%.

C078

CLINICAL FOLLOW-UP OF 378 PATIENTS WITH AUTOIMMUNE HEMOLYTIC ANEMIA: PROGNOSTIC IMPACT OF HEMOGLOBIN LEVELS, AUTOANTIBODY CLASS, AND RETICULOCYTOPENIA AT ONSET ON THE RELAPSE RISK AND OUTCOME

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Autoimmune hemolytic anemia (AIHA) is greatly heterogeneous, from mild/compensated to life-threatening, due to autoantibody class/thermal amplitude and bone marrow compensatory response. Here we studied 378 patients (135 M and 243 F, median age 61 yrs, range 19-100), followed-up for 4.3 yrs (range 0.5-27), classified in warm (w)AIHA (DAT positive for IgG and IgG+C), cold agglutinin disease, CAD (C), mixed (IgG+C with high titer cold agglutinins) and atypical (DAT-, IgA+, wIgM). Anemia was categorized in Hb<6, 6-8, 8-10 and >10 g/dl, LDH expressed as fold upper the limit of normality (ULN) and reticulocytes as absolute count and index. The therapy lines were: steroids, rituximab, splenectomy, immunosuppressors, and transfusions/plasma exchange/erythropoietin. Hb was lower in IgG+C wAIHA and atypical cases (p<0.001), LDH higher in IgG+C wAIHA, mixed and atypical forms (p=0.01), and Hb and LDH values were negatively correlated (r=-0.25,p<0.001)[Table1]. Reticulocytes were lower in CAD, mixed and IgG+C wAIHA (p<0.001) with inadequate reticulocytosis (p=0.01). Moreover, reticulocyte index was lower in cases with Hb<6 g/dL (p<0.001), with inadequate reticulocytosis (87 *vs* 70%, p=0.01).1st line therapy was administered in all cases but 25 CAD. 2nd line was mostly required in IgG+C wAIHA, mixed, and CAD (p=0.005). Ultra-refractory cases requiring 4 or> lines were mixed, atypical, and CAD. Patients with Hb<8 g/dL frequently required a 2nd line (51 *vs* 33%, p=0.004; p=0.03), or 3 or > lines (73% *vs* 26%, p<0.001). The following hazard ratios (HR) emerged from multivariate analysis: 3.2 (95% CI 1.4-7), 2.9 (1.4-6.2), 3.4 (1.6-7.5), for Hb<6, 6-8, and 8-10 g/dL compared to patients with Hb>10. Infections occurred in 14% of cases (mostly mixed AIHA, p=0.02), thrombosis in 10%, and acute renal failure (ARF) in 3% with no relationship with AIHA type/Hb. Evans' syndrome was frequent in mixed or atypical (p=0.04) and in severe forms (74% with Hb<8 g/dL *vs* 26%, p=0.005), and associated with higher relapse risk (HR 2.3, 95% CI 1.4-3.9). Seventy patients died, 12 because of AIHA complications. Mortality correlated with infections (HR 5.8), ARF (HR 7.6) and Evans' syndrome (HR 8.3). In conclusion, we found that anemia severity at onset was the major determinant of relapse risk. The lowest Hb levels were observed in patients with IgG+C WAIHA and atypical cases along with higher LDH levels and inadequate reticulocytosis, advising strict clinical observation in these patients.

Table 1.

TABLE 1	WAIHA (n=193)		CAD (n=109)	Mixed AIHA (n=56)	Atypical AIHA (n=20)
	IgG (n=159)	IgG+C (n=34)			
Hb (g/dL) median (range)	7.3 (2.1-14)	5.8 (2-10.7)	8.2 (4-13.5)	7 (2.9-11.5)	6.2 (3-9)
LDH ULN median (range)	1.7 (0-27)	1.8 (1-5)	1.4 (0-12)	1.9 (1-10)	2 (1-18)
Ret (x10^9/L) median (range)	192 (22-644)	156 (53-495)	122.5 (33-644)	150 (45-310)	202 (29-780)
Inadequate reticulocytosis (n of pts, %)	85 (53%)	23 (37%)	71 (65%)	27 (48%)	11 (55%)
Anemia					
Very severe anemia (Hb<6 g/dL)	44 (28%)	20 (58%)	10 (9%)	18 (32%)	10 (50%)
Severe anemia (6<Hb<8 g/dL)	63 (40%)	10 (30%)	41 (38%)	21 (38%)	7 (35%)
Moderate anemia (8<Hb<10 g/dL)	37 (23%)	2 (6%)	34 (31%)	14 (25%)	3 (15%)
Mild anemia (Hb>10 g/dL)	15 (9%)	2 (6%)	24 (22%)	3 (5%)	0 (0%)
Therapy					
No therapy	8 (5%)	0 (0%)	25 (23%)	2 (3%)	1 (5%)
1 line of therpy (n of pts, %)	151 (95%)	34 (100%)	84 (77%)	55 (98%)	19 (95%)
2 lines of therpy (n of pts, %)	59 (37%)	19 (56%)	51 (47%)	36 (64%)	7 (35%)
3 lines of therpy (n of pts, %)	23 (15%)	6 (18%)	26 (24%)	15 (27%)	4 (20%)
4 or more lines of therpy (n of pts, %)	7 (4%)	0 (0%)	11 (10%)	5 (9%)	2 (10%)
Complications					
Infections (n of pts, %)	23 (14%)	5 (15%)	10 (9%)	14 (25%)	0 (0%)
Thrombosis (n of pts, %)	23 (14%)	7 (20%)	15 (14%)	11 (20%)	2 (10%)
Acute renal failure (n of pts, %)	5 (3%)	2 (6%)	2 (2%)	2 (4%)	0 (0%)
Evans' (n of pts, %)	11 (7%)	4 (12%)	1 (1%)	5 (9%)	2 (10%)
Death (n of pts, %)	31 (19%)	7 20%)	22 (20%)	6 (11%)	3 (15%)
Death for AIHA (n of pts, %)	5 (3%)	2 (6%)	2 (2%)	3 (5%)	0 (0%)

C079

RATE AND REASONS OF 5-AZACYTIDINE DISCONTINUATION AND SUBSEQUENT THERAPEUTIC OPTIONS IN 418 MDS PATIENTS FROM THE ITALIAN MDS REGISTRY OF FONDAZIONE ITALIANA SINDROMI MIELODISPLASTICHE (FISM)

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Azacytidine (AZA) is the current standard of care for patients with high-risk myelodysplastic syndrome (MDS) in Europe. However, about 40% of patients do not respond and most patients loose response within 2 years. Treatment options for MDS patients failing hypomethylating agents therapy are scarce. Only non-selected patients recorded in the MDS registry of Fondazione Italiana Sindromi Mielodisplastiche (FISM) and treated with AZA from January 2009 to June 2014 were considered for the analysis. 418 patients actually received AZA; 269 as 1st

line treatment (64%), 115 as 2nd line treatment (28%), and 34 as a line ≥3rd (8%). Median age was 73 years (range 18-91). At start of AZA therapy IPSS score was low in 14 (3%), int-1 in 97 (23%), int-2 in 183 (44%), high in 67 patients (16%), and not available in 57 patients (14%). Patients received a median of 7 courses of treatment (range 1-63). Median OS was 23 months, OS after discontinuation of AZA was 8 months. Clinical response according to IWG criteria have been reported in 344/418 (82%) patients; 45/344 patients (13%) achieved a complete hematological response, 77 (22%) a partial response, 86 (25%) had stable disease while 136 (40%) did not respond. Response was achieved after a median of 6 cycles. After a median follow up of 16 months (range 7-35) in 37 patients (9%) AZA therapy was still ongoing and in 381 (91%) has been discontinued. Interruption of treatment was due to loss of response in 59 patients (15%), AML evolution in 154 (40%), death in 43 (11%), toxicity or poor compliance in 39 (10%), allogeneic transplant in 12 (3%), other reasons in 22 (6%), not reported in 52 patients (14%). Of the 381 patients who discontinued AZA 15 (4%) were managed with intensive AML-like chemotherapy, 22 (6%) received an allogeneic HSCT, 27 (7%) low-dose chemotherapy, 22 (6%) erythroid stimulating agents ,18 (5%) other treatments and 277 patients (72%) no further treatment or only supportive therapy. Our data confirm that AZA therapy is effective for MDS patients, both with higher and lower IPSS risk disease. Response rate is consistent with what previously reported and median OS is 23 months. The interesting observation is that at 16 months, 91% of patients had discontinued treatment, either for progression or loss of response and only in 10% of cases for reported toxicity. Only 28% of patients received any kind of salvage therapy and overall survival after AZA discontinuation was poor (8 months).

C080

INTERIM RESULTS FROM A PROSPECTIVE OBSERVATIONAL STUDY OF THE "RETE EMATOLOGICA LOMBARDA" (EPOREL1) ON THE USE OF BIOSIMILAR ERYTHROPOIETIN ALFA (HX575) FOR THE TERAPY OF ANEMIA IN "LOWER RISK" MYELODYSPLASTIC SYNDROMES

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Prospective evaluation about the use of biosimilar EPOs for anemia in MDS are lacking. From November 2014 anemic pts with newly-diagnosed "lower risk" MDS afferred to the centres of REL treated with biosimilar EPO participate to the observational multicenter prospective study named EPOREL1 protocol. The primary endpoint is to prospectively assess the response rate of "lower risk" MDS anemic pts treated with biosimilar EPO alfa; the secondary endpoint to validate the prognostic power of Scandinavian Myelodysplasia Group (SMG) score in this setting. MDS were diagnosed according to WHO 2008 criteria and classified according to IPSS and IPSS-R. EPO alfa was administered subcutaneously at the starting dose of 40.000 U/w. EPO was doubled in not responsive pts after 2 mo, reduced at mainteinance dose if hgb >12g/dl and stopped in case of progression or AML evolution, death, major toxicity. Responses were defined by IWG criteria 2006. Forty of 42 consecutive enrolled pts were evaluable. Median age was 77 yrs (65-90); F/M 0,3. Their diagnostic and prognostic characteristics are described in Table. Twelve (50%) pts were transfusion dependent. Median baseline hemoglobin was 8,8 (7,5-10,3) and median endogeneous serum EPO, evaluable in 37 pts (92,5%), was 50 U/L (11-1410). After a median f.up of 11 mo (2-36), 30 pts (75%) responded after a median time of 1 mo (1-10), 12/20 (60%) transfusion dependent pts gained at least 1,5 g/L hemoglobin. EPO dose was incremented to 56-80.000U/w in 19 pts (47,5%) after a median of 2 mo (1-8). No adverse events were reported up to now. 23/27 (85%) pts with SMG score good and 5/9 (55%) pts with SMG score intermediate achieved an erythroid response (p=0.08). One case progressed to "higher risk" MDS after 20 mo; AML evolution was documented in three not responsive cases after a median follow-up of 3 ms (2-6): two pts were off therapy; one case IPSS int1, IPSS-R int, normal karyotype, evolved during treatment. Two pts died: one off-therapy after AML transformation for septic shock, one responsive with previous cardiopathy for congestive hearth failure. To our knowledge this is the largest prospective cohort of MDS pts treatment with biosimilar EPO. The response rate and safety profile of biosimilar EPO alfa is comparable with published data on originator EPO, even in this older MDS cohort. The prognostic power of SMG score isn't confirmed.

Table 1.

WHO 2008	N (%)	IPSS	N (%)	IPSS-R	N (%)
rcud (RA)	7 (17,5)	LOW	24 (60)	VERY LOW	3 (7,5)
rcud- RS	9 (22,5)	INT 1	15 (37,5)	LOW	19 (47,5)
RCMD +/- RS	17 (42,5)	NA	1 (2,5)	INT	16 (40)
RAEB I	6 (15)			NA	2 (5)
MDS/MPN	1 (2,5)				
TOT	40				

Myeloproliferative Disorders 2

C081

A GREATER MUTATIONAL COMPLEXITY CONTRIBUTES TO THE DIFFERENTIAL PROGNOSTIC IMPACT OF DRIVER MUTATIONS IN PREFIBROTIC-PMF

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Background: In primary myelofibrosis (PMF), JAK2/CALR/MPL mutations are prognostically informative, with "triple- negative" (TN) patients (pts) displaying the worst survival (OS). OS was significantly shorter in CALR type2 mutated pts and in those in molecular risk category (HMR). Prefibrotic (P-) and overt (O-) PMF display a distinct clinical and molecular phenotype. *Aims:* To analyze the molecular landscape of WHO2016 diagnosis PMF pts categorized according to their driver mutation status and correlate with outcome. *Methods:* 676 2016WHO PMF were included. NGS analysis was used to genotype a panel of 18 myeloid neoplasm associated mutations genes (NPAMs). *Results:* We analyzed 286 P- and 390 O-PMF pts. P-PMF: 189 were JAK2+ (66.0%), 30 TN (10.5%) and 53 CALR+ (18.5%; 68% Ty1 and 32% Ty2). 94 pts (32.9%) died after a median follow up of 6.2y. More deaths occurred among Ty2 (45.8%), JAK2+ (37.0%) and TN (72.7%) pts compared to Ty1 (20.0%) (P<.0001). Median OS of Ty1 was 27.7y *vs* 22.8y for Ty2, 12.2y for JAK2+ and 2.6 for TN (P<.0001). O-PMF: 277 were JAK2+ (71.0%), 52 TN (13.3%) and 86 CALR+ (22%; 80% Ty1 and 17 20% Ty2). Death occurred in 229 pts (58.7%) after a median follow up of 4.2y. Median OS of Ty1 was 11.2y *vs* 7.5y for Ty2, 5.4y for JAK2+ and 2.2 for TN (P<.0001). HMR status was associated with shortened OS (HR 2.9 in P- and 1.93 in O-PMF; P<.001) as it was the HMR>=2 mutations (HR 7.43 in P- and 2.71 in O-PMF;P<.001). Pts harboring any mutations in NPAMs were similarly distributed among groups in O-PMF: Ty1 60%, Ty2 90%, JAK2+ 65%, TN 73%; exceptions were SRSF2 mutations (n=42) that were 1% Ty1, 12% Ty2, 10% JAK2+, 25% TN (P=.001). The proportion of HMR was significantly higher in TN (56%), Ty2(53%) and JAK2+ (44%) compared with Ty1 (29%;P=.04), as it was the percentage of pts with HMR>=2 mutations (P=.03). In P-PMF, TN harbored significant higher NPAMs (79%) in comparison with Ty1 14%, Ty2 50% and JAK2+ 41% (P=.01); including pts having HMR>=2 mutations (P=.01).In particular TN displayed more mutations in ASXL1 (P<.0001), SRSF2 (P=.03) and NRAS (P=.04). *Conclusions:* Overall, our results confirmed the prognostic advantage of CALR Ty1 mutation in PMF; we found evidence that, in P- but not O-PMF, such difference might be ascribed to a greater molecular complexity of Ty2. Moreover, the dismal outcome of TN pts might be explained by occurrence of greater number of prognostically negative HMR mutations, particularly SRSF2 mutations in both categories.

C082

THROMBOSIS FREE SURVIVAL IN 734 PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (WHO 2016) STRATIFIED ACCORDING TO THE REVISED IPSET-THROMBOSIS SCORE. A REPORT OF THE REGISTRO ITALIANO TROMBOCITEMIE (RIT)

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Background: The Revised International prognostic Score for Thrombosis in Essential Thrombocythemia (R-IPSET-Th) is based on different combinations of 3 parameters: Age >60 years (Age >60), JAK2 V617F mutation (JAK2+), and Prior Thrombosis (PrTh+). *Aim:* To validate the R-IPSET-Th in a cohort of ET patients reclassified according to the WHO 2016 criteria. *Methods:* The ET patients of the web-based Registro Italiano Trombocitemie (RIT) were stratified, according to the R-IPSET-Th score, in 4 thrombotic risk groups: Very Low Risk (VLR: No Age>60, No JAK2+, No PrTh+), Low risk (LR: only JAK2+), Intermediate Risk (IR: only Age>60), High Risk (HR: PrTh+ or Age>60 with JAK2+). The first thrombotic events occurring during the follow-up (ThFUP) were reported (n,%, n/100 pt-yr) for each group, together with thrombosis free survival (TFS, time from diagnosis to the first ThFUP). *Results:* Overall, 734 ET patients were analyzed (females 62%). Data at diagnosis were: Age>60 in 286 (39%), JAK2+ in 417 (57%), and PrTh+ in 126 (17%) patients. Moreover: cardiovascular risk factors (CVRF) in 53%, PLT >1000 x 10^9/L in 17%, and WBC >10 x 10^9/L in 25% of patients. The patients were: VLR 193 (26%), LR 197 (27%), IR 79 (11%), and HR 265 (36%). Their median FUP was 12, 12, 9, and 11 years, respectively. The rates of treatment were: 82%, 92%, 92%, 91% with anti-platelet (AntiPLT) drugs (mainly low dose aspirin); 67%, 61%, 94%, 95% with Cytoreductive drugs (mainly hydroxycarbamide, HC). The ThFUP (n 103, 14%) increased (p<0.001) with the risk score: in VLR (n 15, 8%), in LR (n 20, 10%), in IR (n 12, 15%), in HR (n 56, 21%). The ThFUP/100 pt-yr similarly increased (p<0.01) as follows: 0.60%, 0.79%, 1.61%, and 1.91%, respectively. The TFS progressively decreased (p<0.001) from VLR group to HR group (Figure 1). In detail, the probability of TFS in the 4 risk groups was at 5 years 0.98, 0.97, 0.94, 0.88 and at 20 years 0.85, 0.87, 0.78, 0.54, respectively. An high concordance index (Harrell C) was found (0.82). A comparative stratification according to the R-IPSET-Th score and other thrombotic risk scores (conventional, IPSET, IPSET-Th) was done. *Conclusions:* This study of the Registro Italiano Trombocitemie (RIT) documented that the Revised International Prognostic Score for Thrombosis in Essential Thrombocythemia (R-IPSET-Th) was able to stratify patients in 4 groups with increasing risk for thrombosis during the follow-up (p<0.001).

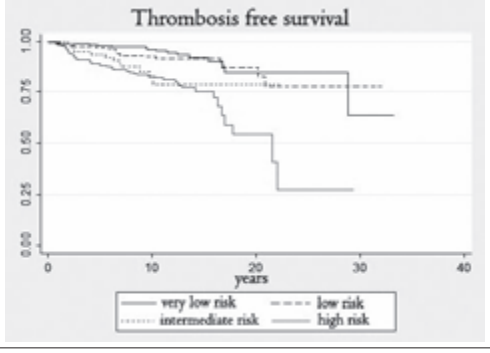

Figure 1.

C083

EFFECT ON CLINICAL OUTCOME OF BONE MARROW RETICULIN FIBROSIS IN 579 PATIENTS WITH POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA

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Background: Polycythemia Vera (PV) and Essential Thrombocythemia (ET) are long term outcome myeloproliferative neoplasm (MPN); however, they could evolve to secondary myelofibrosis-MF or acute leukemia-AL. Prognostic value of bone marrow (BM) fibrosis grading in PV and TE patients is still debated. *Aims and Methods:* We retrospectively analyzed a cohort of 579 PV (n=180) and ET (n=399) patients, diagnosed between 1990 and 2013 in Turin and Bologna, and examined the prevalence and prognostic relevance of BM reticulin fibrosis. Eligibility criteria included the availability of BM samples at diagnosis. BM biopsy sample were reviewed by local pathologist and fiber scoring was performed according to a 3-graded system. Patients with grade 2 or 3 fibrosis were excluded. We evaluated overall survival (OS) using Kaplan Meyer method and HR were estimated with the Cox Model. Cumulative incidence (CI) of MF and AL evolution were estimated considering death from any cause. *Results:*115 (63%) grade 0 and 65 (36%) grade 1 fibrosis and 291 (72%) grade 0 and 108 (27%) grade 1 among PV and ET cases, respectively (p= 0.028) We analyzed effect on clinical outcome separately. PV: at a median follow up of 110 months (IQR:70-170), 5 and 10-years OS were 96% and 87%, respectively. Stratified by fibrosis degree the 5 and 10-years OS were 98% *vs* 90% and 92% *vs* 82% for grade 0 and grade 1 (p 0.076), respectively. CI of MF evolution at 5 and 10-years was 2,8% and 7,2% *vs* 3,8% and 18,7% for grade 0 and grade 1 (p 0.123). CI of AL evolution at 10- year was 4,2% for both grade whereas at 15-years was 4,2% *vs* 19% for grade 0 and 1. ET: at a median follow up of 75 months (IQR:39-120), 5 and 10-years OS were 98% and 90%, respectively. The 5 and 10-years OS were 98% and 90% for grade 0 *vs* 97% and 89% for grade 1 (p 0.358), respectively, when stratified by fibrosis degree. Mutation status was analyzed in 379 TE patients. CI of MF evolution was at 5-years 0,5% and 9% and at 10-years 6,2% and 18% for grade 0 and 1 (p 0.0001). CI of AL evolution at 5 and 10-years was 0% for grade 0 and 13% and 7,3% for grade 1(p 0.096). Grade 0 showed a higher cumulative risk of AL evolution at 15-years (6,9% *vs* 10% for grade 0 and 1) (p=0.096). *Conclusions:* In ET patients grade 1 BM fibrosis seems to correlate with a higher cumulative risk of MF and AL evolution, whereas in PV patients seems to correlate to a trend of higher mortality, even if not statistically significantly.

C084

INCIDENCE OF EARLY THROMBOSIS IN ESSENTIAL THROMBOCYTHEMIA (ET): A PROSPECTIVE ANALYSIS FROM THE PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS (MPN) LATIUM GROUP

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Background: Thrombotic episodes are the major complication in the follow-up of Essential Thrombocythemia (ET), with high morbidity and mortality, as reported in several retrospective studies. At present, however, very few prospective data are available on the early incidence (<4 years from diagnosis) of these complications in ET. *Methods:* To address this issue, we report on 561 consecutive patients [M/F 209/352, median age 66.1 years, interquartile range (IQR) 52.4 – 75.4] with newly diagnosed ET according to WHO 2008 criteria enrolled in the prospective database of our regional cooperative group since January 2011 to December 2015. The main clinical features at diagnosis of the whole cohort are reported in Table 1. *Results:* On the whole, 13 episodes of early thrombotic complications were reported in 561 patients (2.3%) at a median interval from diagnosis of 20.2 months (IQR 11.7 – 36.0): in particular, 8 (61.5%) were arterial (4 cerebral, 1 coronaric, 3 in the lower limbs) and 5 (38.5%) venous (4 in the lower limbs and 1 in the upper limbs). The 4-year cumulative Thrombosis-Free Survival (TFS) of the whole cohort was 96.7% (95%CI 94.8–98.6). Several clinical features at diagnosis (age, gender, Hb levels, WBC and PLT counts, spleen enlargement, JAK-2 V617F mutation and previous thrombotic events) were evaluated for a role in predicting early thrombotic events: only age (p=0.037) and previous thrombotic events (p=0.030) were significant. The 4-year cumulative Overall Survival of the whole cohort was 99.8% (95%CI 99.5–100). *Conclusions:* The incidence of early thrombosis seems low in the first 4 years after diagnosis of ET based on our prospective database: it is worth of note that only age and previous thrombotic events had a predictive role, thus confirming many retrospective reported data and reinforcing the prognostic value of old scoring system for thrombotic risk in ET.

Table 1. Clinical features at diagnosis.

	ET
N° of patients	561
M/F, n° (%)	209/352 (37.6/62.4)
Median age, yrs (IQR)	66.1 (52.4-75.4)
Median Hb at diagnosis, g/dl (IQR)	14.0 (12.8-15.0)
Median WBC at diagnosis, x 10^9/l (IQR)	8.60 (7.17-10.8)
Median PLTS at diagnosis, x 10^9/l (IQR)	715 (597 – 888)
V617F JAK-2 positive, %	66.0
Previous thrombosis, n° (%)	94 (17.0)

C085

EPIDEMIOLOGY, OUTCOME AND RISK FACTORS FOR INFECTIOUS COMPLICATIONS IN MF PATIENTS RECEIVING RUXOLITINIB. A MULTICENTER STUDY ON 446 PATIENTS

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Ruxolitinib significantly ameliorates disease-related splenomegaly and constitutional symptoms in myelofibrosis pts.In prospective studies, around 50% of pts experienced at least an infection including opportunistic and unusual infections,probably due to its immunesuppressant activity. Clinical and laboratory data of MF pts treated with RUX were retrospectively collected from the database of 22 Centers, with the aim to investigate epidemiology, outcome and risk factors for infections.Overall, 446 pts received RUX between Jun 2011 and Nov 2016.At RUX start the clinical features were:age 68 years (27-89), ≥65y, 62%; male, 57%;Hb, 10.7 g/dL (4.7-16.7); Hb <10g/dL, 36%;PLT, 250x10^9/L (33-1887); PLT <100 10^9/L, 10%; enlarged spleen, 96%; spleen length ≥10cm, 62%; constitutional symptoms, 54%.IPSS was intm-1 (16%), intm-2 (47%), high (37%);unfavourable karyotype 8%,previous infections 8%.JAK2V617F mutation 81%.After a median RUX exposure of 19 months (range, 1-56),118 pts (26%) experienced 154 infectious events (grade 3, 33%, fatal 10%),for an incidence rate of 16.4 cases for 100 pts/year.The rate of infections tended to decrease over time:51% occurred within 6 months of therapy,16% between 6-12 months, 8% between 12-18 months (p<0.0001).Respiratory tract infections were more frequently observed (82 events, 53%).Etiological agents were isolated in 15 cases (10%): bacteria in 9 cases (gram+ 78%, gram- 22%) and fungi in 3 cases;Mycobacterium tuberculosis was isolated in 3 cases, none of them was on isoniazid prophylaxis. Herpesvirus reactivations occurred in 13 cases (9%). Seven pts (2%) were on acyclovir for previous herpes virus reactivation. Overall, 1% of patients were HCV IgG positive; 9% carried occult HBV infection, 50% of them received lamivudine as prophylaxis. No patient reactivated hepatitis virus infection. Among baseline features, age ≥65 years at RUX start (p=0.001), previous infection (p=0.002), high IPSS (p=0.005),CCI >3 (p=0.02), smoke (p=0.014) and diabetes (p=0.05) significantly correlated with higher infectious risk.In multivariate analysis, age ≥65 years (HR 2.23 CI95%1.27-3.92), previous infection(HR 2.03 CI95%1.06-4.5) confirmed their negative prognostic association.This real-life study reports an incidence of infections (26%) that is inferior to previous prospective studies, probably due to improved infectious screening and prophylaxis.Pts with older age and previous history of infections were found to be at increased infectious risk.

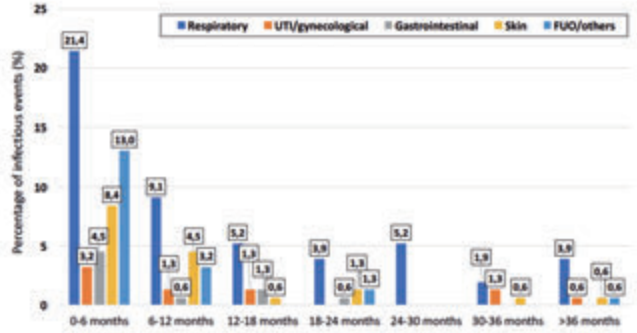

Figure 1. Clinical characteristics of infectious complications.

Transplantation 2

C086

HIGH PROGNOSTIC VALUE OF PRE-SCT MOLECULAR MINIMAL RESIDUAL DISEASE ASSESSMENT BY WT1 GENE EXPRESSION IN WT1 POSITIVE AML TRANSPLANTED IN CYTOLOGIC COMPLETE REMISSION

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Introduction: We analyzed the outcome of allogeneic Stem Cell Transplantation (allo-SCT) in AML patients according to molecular Minimal Residual Disease (MRD) at the pre transplantation (pre-SCT) workup, assessed by the quantitative expression evaluation of the panleukemic marker Wilms' tumor gene (WT1), according to LeukemiaNET validated method. *Patients and Results:* 122 consecutive AML patients, WT1 positive at diagnosis, received allo-SCT while in cytologic Complete Remission (cCR), between 2005 and 2016, at our Center. The median age at SCT was 53 years (18-70). The quantitative analysis of the WT1 gene expression (bone marrow samples) was available in 100% cases both at diagnosis (100% overexpressing WT1 with a mean of 8607±8187 copies/104 Abelson) and immediately before allo-SCT (81/122-66% MRD-WT1-negative and 41/122-44% MRD-WT1 positive cases at the pre-SCT workup). We evaluated post-SCT Overall Survival (OS), Disease Free Survival (DFS) and Relapse Rate, according to MRD-WT1 pre-SCT status. Both post-allo-SCT OS and DFS were significantly better in patients who were MRD-WT1 negative (WT1<250 copies) at the time of SCT compared with those who were MRD-WT1 positive (WT1>250 copies), with a median OS and DFS not reached in the MRD-WT1 negative group and 9 and 8 months, respectively, in the MRD-WT1 positive group (OS log-rank p<0.0001; hazard ratio [HR]=3.9, 95% confidence interval [95% CI]=2.0-7.38; DFS log-rank p<0.0001; HR=3.73, 95% CI=2.0-6.72). The relapse rate after allo-SCT was 15% (12/81) in pre-SCT MRD-WT1 negative cases and 44% (18/41) in MRD-WT1 positive cases (p=0.00073). At univariate analysis, MRD-WT1 negativity before allo-SCT and grade <2 acute GVHD were significant prognostic factors for improved OS and DFS. However, at multivariate analysis, MRD-WT1 negativity before allo-SCT was the only independent prognostic factors for improved OS and DFS. *Conclusions:* These data show that pre-allo-SCT molecular MRD evaluation through WT1 expression is a powerful predictor of post-SCT outcome (OS, DFS, relapse rate) and patients with both cCR and a MRD-WT1 negativity before allo-SCT have a very good outcome with a very low relapse rate and better survival. The pre-SCT MRD-WT1 stratification in AML is a valuable tool to identify patients, transplanted in cCR, who are at high risk of relapse and who could be considered for conditioning regimen intensification and/or for post-SCT preemptive strategies (DLI, azacitidine or new target drugs).

C087

A SCORING SYSTEM BASED ON AGE, CMV STATUS AND PRE-TRANSPLANT LEVELS OF IGA AND IGM PREDICT INFECTION-RELATED MORTALITY AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Infection-related mortality (IRM) accounts for a substantial component of non-relapse mortality (NRM) after allogeneic hematopoietic stem cell transplantation (allo-HSCT). No scores have been developed to predict IRM before allo-HSCT.Pre-transplant clinical and biochemical data were collected in a study cohort of 607 adult patients receiving allo-HSCT from January 2009 to February 2017. Median follow-up was 43 months (range 1-85). Acute leukemia was the main indication to transplant, accounting for 60% (n=356) of patients; the majority of them received an alternative-donor transplant (44% a HLA-haploidentical, 37% a matched unrelated donor). Forty-seven percent (n=277) of

patients had advanced diseases. In a training set of 273 patients, ROC curve analysis were used to define the optimal cut-off predicting IRM for continuous biochemical variables. Multivariate analysis revealed age >60 yrs (P=0.003), CMV host/donor serostatus different from negative/negative (P<0.001) and pre-transplant levels of IgA <1.11 g/L (P=0.004) and IgM <0.305 g/L (P=0.028) as independent predictors of increased IRM, independently from donor type or disease status at HSCT. Based on these results, a 3-tiered weighted IRM prognostic index was elaborated and subsequently validated in a retrospective (n=219) and in a prospective (n=115) cohort of patients. According to the score, patients were assigned to 3 different risk classes. The score significantly predicted IRM in the training and in the retrospective and prospective validation sets (P<0.001, P=0.044 and P=0.011). In the training set, 100-day IRM was of 5%, 11% and 16% for low, intermediate and high risk groups, respectively; in the retrospective set was of 7%, 17% and 28%, respectively and in the prospective set 0%, 5% and 7%. Interestingly, this score predicted also the overall survival (P<0.001, P=0.041 and P=0.023, respectively) (Figure 1). Patients belonging to the high-risk class had a significantly lower CMV-free survival (P=0.001) and showed a persistent impaired IgA and IgM recovery after HSCT compared to low-risk patients. In conclusion, this composite scoring system based on pre-transplant data clearly identify patients at higher risk of subsequent fatal infections, thus promoting personalized intensive active surveillance strategies, pre-emptive therapies and immune-intervention approaches to improve the overall HSCT outcome. An Italian multicentric study for the external validation of these results is currently on the way.

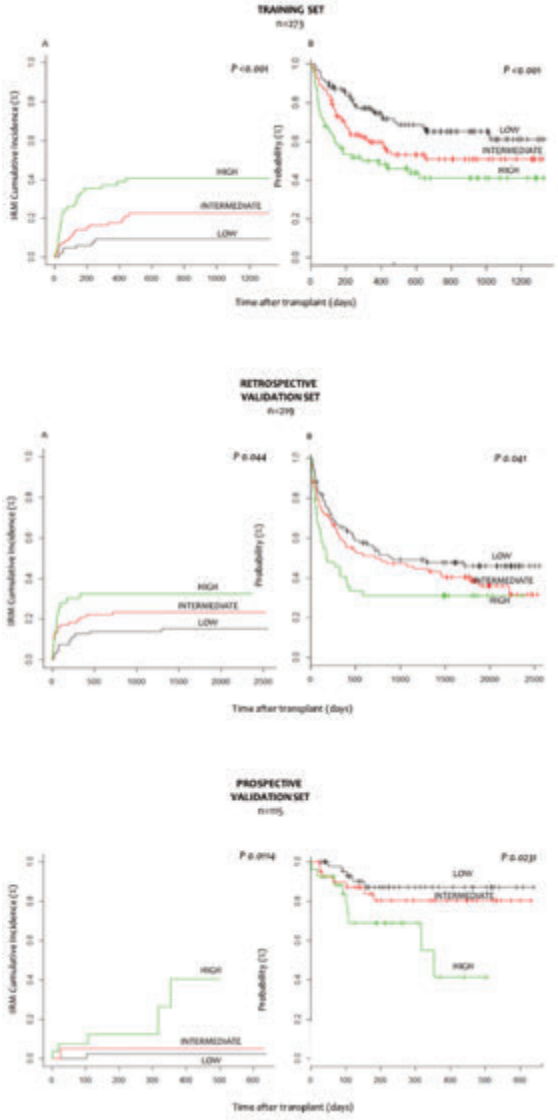

Figure 1. IRM (A) and OS (B) in the 3 sets according to the IRM score.

C088

CONFRONTO TRA REGIME DI CONDIZIONAMENTO BEAM E FEAM IN PAZIENTI AFFETTI DA LINFOMA SOTTOPOSTI AD AUTOTRAPIANTO DI CELLULE STAMINALI EMOPOIETICHE: UNO STUDIO MULTICENTRICO RETROSPETTIVO DELLA FONDAZIONE ITALIANA LINFOMI (FIL)

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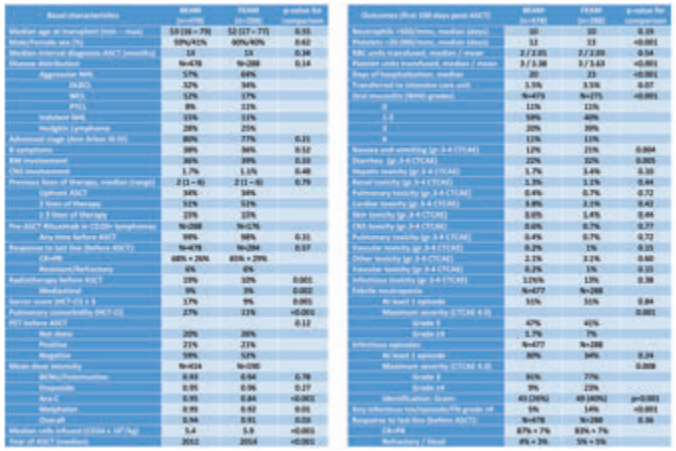
While BEAM is considered the standard conditioning regimen in Europe for lymphomas, substitution of BCNU (due to shortages) with Fotemustine (FEAM) became increasingly popular, although no studies compared BEAM to FEAM. This is a retrospective observational study aimed to compare the safety and efficacy of FEAM with respect to BEAM. We collected and analyzed 766 ASCTs performed for lymphomas in 16 Italian centers from 2008 to 2015: selection for BEAM or FEAM conditioning hinged on BCNU availability and not on clinical characteristics. Median age was 53 years; 41% were females. Indications for ASCT were: aggressive NHL in 59% (DLBCL 32%, MCL 14%, PTCL 9%), indolent NHL in 13% (FL 12%) and HL in 27%. ASCT was performed upfront in 34%, after 1 salvage treatment in 51%, after ≥3 lines of therapy in 15%. Pre-ASCT response status was: CR in 67%, PR in 27%, resistant disease (RD) in 6%. BEAM (n=478) and FEAM (n=278) groups were not significantly different for most of basal characteristics, but Sorror score was higher in BEAM (reported in Table 1); ASCT timeframe was earlier for BEAM (median ASCT year: 2011 BEAM *vs* 2014 FEAM, p<0.001).

Table 1.



Overall dose intensity for conditioning was lower in FEAM, owing to more Ara-C and Melphalan reductions. Oral mucositis (grade ≥3) was more severe in FEAM (50% *vs* 31% BEAM),as well as diarrhea, nausea and vomiting, while other severe toxicities (CTCAE grade ≥3) did not differ. Febrile neutropenias (FN) and severe infectious events with microbiological identification (IE) occurred at a similar rate but were more severe for FEAM (grade 4 FN: 6.6% FEAM *vs* 1.5% BEAM,p=0.001; grade ≥4 IE:23% FEAM *vs* 9% BEAM, p=0.008); FEAM had more Gram- IE (40% *vs* 26% BEAM, p=0.001). Response status did not differ at day 100 post-ASCT (CR 84%),and neither at last follow-up (continuous CR 62%). Overall survival (OS) was not different (p=0.14; at 2 years: BEAM 87% *vs* FEAM 84%),and neither were non-relapse mortality (BEAM 2.5% *vs* FEAM 4.7%,p=0.17) or relapse incidence (BEAM 10% *vs* FEAM 15%,p=0.13) at 1 year. However,the FEAM group had inferior progression-free survival (p=0.02; PFS at 1 year: BEAM 87% *vs* FEAM 80%). Substitution with Fotemustine did not increase general toxicities or infectious episodes, neither significantly worsened OS; however, we observed increased gastrointestinal toxicities, more severe IE (probably due to higher incidence of Gram-sepsis) and reduced PFS. Fotemustine use does not seem justified in conditioning, if not for easier supply.

C089

PREDICTING CHRONIC GRAFT VERSUS HOST DISEASE AND MORTALITY ON DAY +100 AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: In a previous study, we have shown that chronic graft-*versus* host disease (GvHD) and transplant related mortality (TRM) can be predicted by three laboratory values on day +100: platelets count (Plt), serum cholinesterase (CHE), gamma-glutamyltransferase (GT). The aim of this study was to confirm this result in a larger number of patients. *Patients and methods:* The patient population consisted of 1546 consecutive patients with hematologic disorders allografted between 2001 and 2016, alive on day +100 and free of cGvHD. The donor was an HLA identical sibling (n=652) or an alternative donor (n=894). The median follow-up was 1317 days (range 100-9709). In multivariate Cox analysis, Plt, CHE and GT remained significant. Moreover, serum albumin (ALB) and serum immunoglobulin A (IgA) were identified as additional indicators. For each laboratory item, the day +100 cut-off threshold was chosen using ROC curve test and a score of 1 was given for Plt <109x10^9/L, CHE <2813 IU/ml, GT >136 IU/L, ALB <4 mg/dl and IgA <72 mg/dl. Three prognostic groups were identified: low risk for none or 1 negative predictors (n=590), intermediate risk for 2 or 3 negative predictors (n=644) and high risk for 4 or 5 negative predictors (n=312).

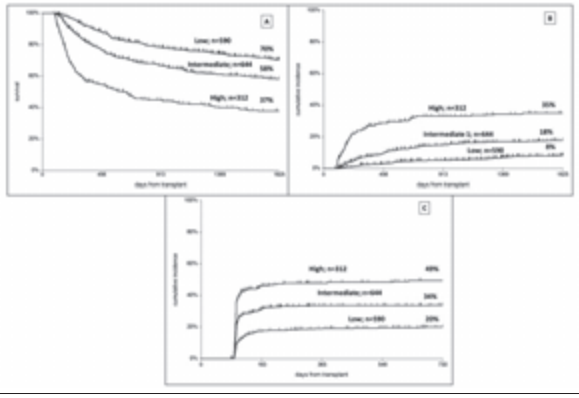

Figure 1. Post-transplant outcomes according to the three risk groups: A) 5-ys OS; B) 5-ys TRM; C) 2-ys cGvHD.

Results: The three risk groups identified patients at different risk of five-years Overall Survival (70% *vs* 58% *vs* 37%, p<0.001; Figure 1A), of five-years Transplant Related Mortality (8% *vs* 18% *vs* 35%, p<0.0001; Figure 1B), and of cGvHD occurrence within the first two years (20% *vs* 34% *vs* 49%, p=<0.0001; Figure 1C). The predictivity of the risk groups on cGvHD (moderate/severe) occurrence were confirmed also stratifying patients for year of transplant (p<0.0001), donor type (p<0.0001), disease status at transplant (p<0.0001) and recipient age (p=0.01). No statistically significant difference was seen between the three risk groups and Relapse-related mortality. *Conclusions:* The use of 5 simple laboratory tests (Plt, CHE, GT, Albumin and IgA) on day +100 after an allograft, predicts cGvHD and mortality, irrespective of donor/patient and disease variables. Prospective trials to prevent cGvHD and mortality in high risk patients, may include immune-modulation with or without intensified infection prophylaxis.

C090

LOW EARLY DOSES OF ATG FOR GVHD PREVENTION IN UNRELATED STEM CELL TRANSPLANT

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Introduction: ATG significantly reduces the risk of cGVHD both in unrelated and in HLA identical sibling stem cell transplants (SCTs). In a prospective study pts undergoing an allogeneic unrelated SCT after a myeloablative regimen were randomized to receive or not 60 mg/kg ATG-Grafalon reporting a significant reduction of cGVHD without increase of relapse and no differences in OS and DFS. A successive study didn't confirm those results (significant reduction of acute and chronic GVHD, but poorer survival mainly due to higher relapse probability in the ATG arm). These conflicting data may also depend from differences in the dose and the timing of ATG.

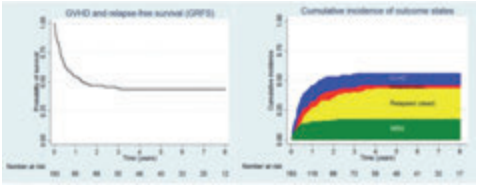

Figure 1.

Patients and methods: We report a large (190 SCT) retrospective monocentric analysis on low ATG doses (15-25 mg/kg for BM according to the degree of HLA matching and 30mg/kg for all PBSC SCT) given early (from day -6 to -2). *Results:* Pts in the study, undergoing SCT between 2005-2015, were AML (n=112, 59%), ALL (n=57, 30%), HR MDS (n=21, 11%), CR1 (n=111, 66%) >CR2 (n=31, 18%), active disease (n=27, 16%) for AL. Median age was 46 (range 18-66). Myeloablative conditioning were BU-Cy120 (n=71, 37%), Bu-Flu-Thio (n=62, 33%), Cy-TBI (n=20, 11%). PBSC was used in 42% (n=80). SCT were performed from HLA 10/10 identical URD (n=62, 33%), 9/10 (n=91, 48%), 8/10 (n=30, 16%) <8/10 (n=7, 4%). Median follow-up was 51.4 months. Gr.2-4 aGVHD was 26%, gr.3-4 aGVHD 9%; cumulative incidence (CI) of cGVHD of any severity was 23%, for mod/sev cGVHD 14%. The organs mostly involved by cGVHD were skin (14.7%), mouth (13.7%), eyes (12.1%) and lung (7.9%); the proportion of patients showing a severity of organ involvement >2 according to NIH criteria was 8.4%, 2.6%,1.6% and 3.2% respectively. The 3-yr CI of relapse and NRM was 26% and 18% respectively. The 3-yr overall and disease-free survival were 60% (95%CI: 52-67%) and 56% (95%CI: 51-68%). The GVHD (aGVHD gr.3-4 and mod/sev cGVHD) and relapse free survival (GRFS) of the entire population (Fig 1) was 44% at 3 years (95%CI: 37-52%). For pts in CR1-2, cGVHD (any severity), GFRS and OS at 3 years were 23%, 50% and 64% respectively. *Conclusions:* Low and early administration of ATG effectively prevents acute and chronic GVHD without increasing relapse, resulting in really convincing GRFS, even for less than 10/10 matched URD SCT. These data suggest a possible alternative to the standard doses actually used in MUD transplant, to be tested in a contest of a prospective randomised study.

Acute Leukemias 3

C091

MINIMAL RESIDUAL DISEASE ASSESSMENT IN ACUTE MYELOID LEUKEMIA WITH MUTATED NPM1: PROSPECTICAL EVALUATION AND MRD DIRECTED THERAPY

F. Guolo, P. Minetto, M. Clavio, C. Di Grazia, N. Colombo, A.M. Raiola, D. Guardo, E. Coviello, F. Ballerini, M. Miglino, E. Angelucci, A. Bacigalupo, R.M. Lemoli, M. Gobbi

IRCCS AOU San Martino-IST

Acute Myeloid Leukemia with mutated NPM1 (NPM-AML) is considered a favorable prognosis entity and allogeneic stem cell transplantation (HSCT) in first complete remission (CR) is not recommended. NPM1-based minimal residual disease (MRD) assessment is widely applied and the reoccurrence of MRD positivity is invariably associated with hematological relapse. No data are at the moment available on the usefulness of salvage therapy delivered at the time of molecular relapse. Our study aims to standardize the definition of molecular relapse and to evaluate the efficacy and feasibility of MRD-directed therapy. Thirty-six consecutive younger patients with NPM-AML intensively treated in our center from 2004 to 2014 were included. After completion of consolidation chemotherapy, NPM-1-based MRD assessment was performed on bone marrow (BM) samples every 3 months for 5 years. If MRD positivity was detected, a second analysis was repeated in 15 days. Before 2015, patients experiencing hematological relapse (HR) received two courses of salvage chemotherapy (MEC) and then proceeded to ASCT. From 2015, salvage treatment was delivered at the time of molecular relapse, and consisted of only one course of MEC. Before 2015, among 36 monitored patients, 13 showed HR. All relapsing patients showed NPM MRD reoccurrence prior to HR. Median time from the NPM MRD reoccurrence and HR was of 4.5 months. MRD relapse was defined as the confirmed reoccurrence of NPM1 mutation, with a total increase of NPM1 expression levels of at least 2 logarithms (i.e at least from 0 to $100/10^4$ Abl). All patients with HR received 2 courses of MEC. Second CR rate was 8/13 (62%) with one patients dying for therapy-related toxicity. Complete NPM MRD clearance was achieved in 4/13 patients (31%). From 2015, 4 consecutive patients have been treated with MRD-directed therapy. Hematological toxicity was significantly lower than that observed in patients treated for HR. Pre and post therapy disease burden, assessed by NPM levels, was significantly lower than in patients treated for HR ($p < 0.001$, Figure 1). All 4 patients were able to achieve a complete MRD clearance before HSCT and are alive and in MRD neg CR. Our preliminary data show that MRD-directed therapy is feasible and reduces the toxicity of salvage treatment. It is also highly effective, increasing the proportion of patients achieving MRD complete response, which deeply affect the outcome after HSCT.

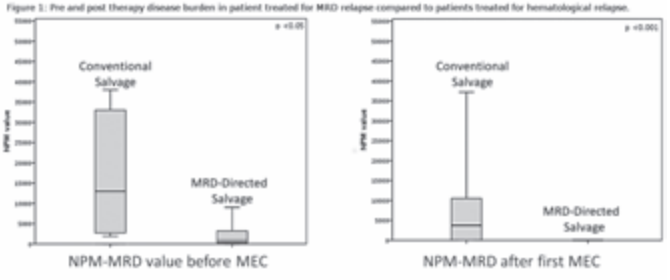

Figure 1.

C092

NEXT GENERATION SEQUENCING (NGS) CONFIRMED THE ADVERSE CLINICAL IMPACT OF RUNX1 MUTATIONS IN A SUBSET OF ACUTE MYELOID LEUKEMIA NORMAL KARYOTYPE PATIENTS NEGATIVE FOR RISK DEFINING GENES MUTATIONS AND HOMOGENOUSLY TREATED WITHIN A CLINICAL TRIAL (NILG AML 02/06)

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Recently, the European LeukemiaNet (ELN) AML guidelines included new molecular alterations, such as RUNX1, ASXL1 and TP53 mutations for a better AML risk stratification. We have verified the clinical impact of these and other molecular alterations in a cohort of 36 normal karyotype AML patients negative for the risk defining FLT3, NPM1 and CEBPA gene mutations and homogenously treated within the clinical trial (NILG AML 02/06). We performed the analysis of 54 myeloid disorders related genes using a commercial, amplicon-based next generation sequencing (NGS) approach (Illumina). Sequencing data were aligned to the reference genome (Human hg19) and variant identification was performed by VariantStudio software (Illumina). The median coverage for the 568 amplicons generated for the 54 analyzed genes was 11341±7199 (range 0-70836). Only 34 out of 54 analyzed genes were mutated in at least one sample. A median of 3 mutations (range 1-7) were found in each patient for a total of 152 mutations. The types of mutation were mostly missense (88) and frame-shift (36). Other mutations were nonsense (13), splicing variants (8) and non frame-shift small indels (7). The most common mutated gene was RUNX1 which was altered in 44% of patients (16/36) followed by ASXL1, SRSF2 and BCOR (22% each), STAG2 (19%), DMT3A and IDH2 (16% each), TET2, SF3B1, BCORL1 and ZRSR2 (14% each), CSF3R, NRAS and NOTCH1 (11% each). Other genes were altered with a frequency inferior to 10%. Mutations of RUNX1 gene did not associate with other gene alterations or clinical/biological characteristics, the "de novo" presentation (24/36) and clinical remission (CR) achievement. Patients with mutated RUNX1 had an inferior overall survival (OS) compared to unmutated patients of this cohort (p=0.0197), while the presence of NRAS alterations interfered with CR achievement (OR=0.06, 0.01-072 95%CI). In conclusion, the data obtained in this cohort of 36 NK AML patients negative for FLT3, NPM1 and CEBPA aberrations showed an incidence of RUNX1 gene mutation higher than previously reported in unselected AML patients. RUNX1 mutations confirmed its adverse prognostic value in this homogenously treated AML patients group whereas NRAS mutations impaired CR achievement, underlying the usefulness of identifying these alterations at diagnosis. NGS approach demonstrated a valid tool for massive sequencing analysis although some issues remain open, such as GC rich genes amplification and long indels proper alignment.

C093

CHROMOTHRIPSIS IN ACUTE MYELOID LEUKEMIA: BIOLOGICAL FEATURES AND IMPACT ON SURVIVAL

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Introduction: Chromothripsis (chrt) has been associated with complex karyotype (CK), genomic instability and aggressive disease in various cancers. Our study describes incidence at diagnosis of chrt in a homogenous cohort of Acute Myeloid Leukemia (AML) patients (pts), impact of chrt on prognosis, and molecular mechanisms associated with this phenomenon. *Patients and Methods:* Samples and data at diagnosis from 395 adult AML pts (M3 excluded) were collected from 3 Institutions. Classical cytogenetics and microarray analysis (SNP 6.0 or Cytoscan HD Arrays, Affymetrix) were performed in all samples. Data were an-

alyzed by R Core Team, chrt was assessed by a custom algorithm based on CTLP Scanner. Overall survival (OS) was analyzed by Kaplan-Meier method and Mantel-Cox test. *Results:* Twenty-six out of 395 pts showed chrt according to Korbel and Campbell's criteria. CTLP Scanner detected chrt mostly on chromosome 12, 17 and 5. The macroscopic chromosomal aberration most associated with chrt was 5q loss (pval<.001, OR 43.6). By FISH and CBA, chrt was associated with marker, derivative and ring chromosomes. Pts with chrt presented a higher mean of Copy Number (CN) Alterations than pts without chrt. CN losses and deletions were enriched for pathways and genes differentially altered between chrt-positive and chrt-negative pts. Examples of genes significantly lost and deleted (pval≤.0001) associated with chrt were HDAC3, PIK3R1, FANCA, WNT5A, MLH1, GATA2. Chrt was associated with higher age, CK, lower WBC count, TP53 loss and mutations (p<.001) and mutually exclusive with FLT3 and NPM1 mutations. Pts with chrt showed a worst OS (120 *vs* 494 days for pts without chrt, p<.001), even when censored for HSCT and when comparing only pts with HR features according to ELN2017 risk stratification, HSCT censored (120 *vs* 211 days respectively, p=.022). Chrt was a consistent risk factor in COX HR model built in HR group considering chrt, secondary AML, induction therapy, FLT3 and NPM1 mutation as categorical variables, and age of diagnosis (HR 2.070, 95% CI 1.167-3.672, p=.013). *Conclusions:* Our work defines for the first time the clinical and biological implications of chrt in AML and shows the usefulness of SNP arrays in defining groups of pts with poor prognosis, which could be candidate to novel target therapies. Acknowledgement ELN, AIL, AIRC, PRIN, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project, HARMONY project. MCF, GM equally contributed*

C094

PHASE 1B RESULTS OF IDASANUTLIN+CYTARABINE (ARA-C) IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS (PTS)

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Background: Idasanutlin is a potent, oral MDM2 antagonist. A Phase 1b study was conducted to assess the safety and efficacy of idasanutlin+ara-C in relapsed/refractory (R/R) AML pts. *Aims:* The primary endpoint was to identify the maximum tolerated dose and/or recommended dose and safety profile of idasanutlin+ara-C. *Methods:* In the dose escalation phase, R/R AML pts or those not considered candidates for standard induction therapies were treated with escalating doses of the initial idasanutlin formulation daily x 5 days (d)+ara-C 1 g/m^2 x 6d. After identification of the recommended dose, an expansion cohort in R/R AML pts treated with ≤2 prior regimens was enrolled. A bridging arm was added to characterize the safety and PK of a spray dried powder (SDP) formulation of idasanutlin. *Results:* A total of 76 pts were treated with the combination therapy. Dose escalation patients (n=23) were treated with 400 mg qd (n=10), 400 mg bid (n=7), or 600 mg bid (n=6) of idasanutlin with ara-C. The recommended dose was 600 mg bid. Twenty-one pts were subsequently treated in an expansion cohort with 600 mg bid idasanutlin+ara-C. Thirty-two pts were enrolled in the bridging arm with either 300 mg bid (n=19) or 400 mg bid (n=13) of SDP idasanutlin+ara-C. The CR proportion was 25% (19/75 pts); the CRc proportion was 29% (22/75 pts), and the CR+CRp+CRi+MLFS proportion was 33% (25/75). Median duration of response is ~6.4 months (range 1.1 to 11.9 mos). Five pts remain in CR and continue in the 1 yr follow up period; 4 pts were in CR at the final visit ~1 yr from start of treatment. MDM2 positivity in CD45dim AML blasts by flow cytometry suggests that higher levels of MDM2 expression are associated with response (p=0.00049). TP53 mutation status was not associated but did trend with response (p=0.08) as predictive/prognostic association derives from negative predictive value of the small proportion of mutant patients. By contrast, MDM2 protein expression by flow cytometry displayed pronounced association with CRc when analyses were restricted to TP53 WT-only patients (p=0.0021). *Conclusions:* Treatment with idasanutlin+ara-C resulted in durable CRs. Five of the 22 pts who achieved a CRc (23%) proceeded to transplant following therapy. Biomarker data suggests that identifying pts in which TP53 activity may be therapeutically enhanced may provide for improved outcomes. A Phase 3 trial is open and accruing.

C095

GENE EXPRESSION PROFILING IDENTIFY NEW ADULT ACUTE LYMPHOBLASTIC LEUKEMIA SUBGROUPS

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Background: Although the remarkable progress made in the treatment of Acute lymphoblastic leukemia (ALL) in children and, with less efficacy, in adults, several ALL subtypes continue to have a poor prognosis. Consequently, there is a need in improving the molecular dissection of subtypes, identifying genetic alterations that predict the risk of treatment failure and developing novel and targeted therapies. Patients (pts) without abnormalities are collectively referred to as B-other ALL. *Aims:* Is to focus on adult B-other ALL in order to define and assess biomarkers in this subgroup to test new drugs. To also find a new target drug in Ph+ or in a Ph+ subgroup to support tyrosin kinase inhibitor treatment especially in relapsed/refractory patients. Patients and *Methods:* Gene Expression Profiling (GEP; HTA 2.0 Affymetrix) were performed on 51 B-other ALL Ph-, 25 B-ALL Ph+ at different time point of the disease and on 7 mononuclear cell of healthy donors. Data were normalized and analyzed with the Expression Console and the Transcriptome Analysis Console (TAC) Software (Affymetrix). *Results:* Comparing GEP of Ph- B-other and Ph+ to donors we found some shared top upreg genes to focus on (*e.g.* EBF1, CD19, BLNK, PDLIM1, PXDN, NAV1, CTGF, LEF1, CD200, CRLF2). In Ph- and Ph+ ALL GEP top upreg gene analysis we identify a well-defined 3-clusters-subdivision (Fig 1) characterized by CTGF, CRLF2 and CD200 expression. We started to analyzed data of one of these 3 genes: CTGF. Ph- B-other and Ph+ *vs* donors comparisons CTGF appeared very significantly upreg (ANOVA p Value: 0.0018 and 0.000084 respectively).

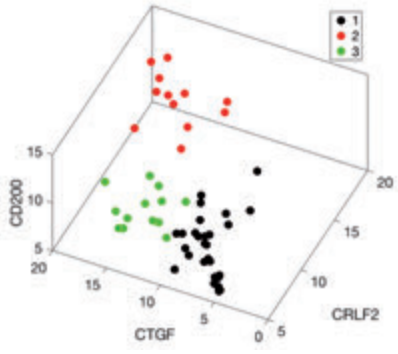

Figure 1. Clustering of Ph+ B-other and Ph+ ALL Gene Expression.

Within Ph- group we can distinguish 2 subgroups with different CTGF expression; one has a significant higher CTGF expression (ANOVA p Value: 0.000056 and 0.02), thing that we couldn't evaluate

in a first Ph- global analysis. *Conclusions:* Based on Ph+ and Ph- B-other GEP we identify 3 new cluster defined by 3 top upreg genes.: CTGF is highly expressed in Ph+ and Ph- ALL, but in B-other we distinguished 2 different expression profiles. CTGF upregulation has been associated with poor outcome in adult ALL pts. Although several studies have been associated CTGF expression with cases of B-ALL, none of them clarified the correlation between its expression and different B-ALL subtypes (also in B-other). We are now testing a CTGF inhibitor to evalue this issue. ELN, AIL, AIRC, PRIN, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project; progetto HARMONY.

Non Hodgkin Lymphomas 3

C096

PET/CT-GUIDED BIOPSY FOR THE DIAGNOSIS OF LYMPHOMA

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Biopsy of affected tissue is required for lymphoma diagnosis at onset and relapse and to plan adequate treatment. Open incisional biopsy is traditionally the method of choice, with an accuracy of approximately 100%. Nevertheless, it requires hospitalization, availability of an operating room and sometimes general anesthesia and is associated with several drawbacks (morbidity, surgical complications, tumor contamination of surrounding tissues). The development of ultrasound and computed tomography (CT)- guided biopsies has almost overcome these disadvantages. However, a variable proportion of non-diagnostic procedures is reported, leading to an accuracy ranging between 50% and 80%. Functional imaging, such as fluorodeoxyglucose (FDG) positron emission tomography (PET)/CT, is a procedure which can potentially drive biopsy to the most metabolically active area within a lymph node or extranodal masses which sometimes show no morphologically detectable changes on CT scan. One hundred patients with suspect lymphoma at onset or relapse are expected to be enrolled in 3 years, provided they show FDG-avid findings. Patients are excluded if pregnant, breastfeeding or in case fine-needle PET/CT-guided biopsy is contraindicated. Diagnostic accuracy will be compared to published data concerning conventional imaging. Specimen adequacy will also be evaluated. The trial is supported by the Italian Association for Cancer Research (Progetto AIRC IG 2015 Id 17781). Data are available for the first 32 patients. Thirty-four procedures have been performed: 3 (8.8%) were interrupted because of pain but could be successfully repeated in 2 cases. Biopsy target was lymph node in 19 cases and extranodal site in 13 (bone in 8 cases, soft tissue in 3, liver and kidney in 1 each). Median SUVmax of target lesions was 11.5 (4.9-37.7). Insufficient samples were obtained in 9.7% of cases (3 out of 31 successful procedures), whereas in all other instances the tissue was considered adequate to formulate a diagnosis (table). Mean sample length was 10 mm (standard deviation ± 6 mm). The mean amount of affected tissue in collected samples was 56% (± 33%) and the mean proportion of fibrosis/bone was 37% (± 32%). No severe adverse events were reported during or after each procedure. Patients can benefit from a minimally invasive procedure which allows a timely and accurate diagnosis of lymphoma at onset or relapse. Cost and time savings will be evaluated once enrolment is fully completed.

Table 1.

Diffuse large B-cell lymphoma	10
Follicular lymphoma	7
Metastases of carcinoma	3
Hodgkin lymphoma	2
Normal tissue/Inflammation	2
Anaplastic large T-cell lymphoma	1
Acute lymphoblastic leukemia	1
Marginal zone lymphoma	1
Mantle cell lymphoma	1

C097

EFFECTIVENESS AND SAFETY OF IBRUTINIB IN RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA TREATED IN ITALY ACCORDING TO THE IBRUTINIB NAMED PATIENT PROGRAM: THE REAL LIFE RESULTS

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Although a typical presentation as an indolent lymphoma, without systemic symptoms and with a good performance status, the mantle cell lymphoma (MCL) is an aggressive one, hardly curable with standard chemo-immunotherapy. Although current approaches to MCL, including newer agents and autologous stem cell transplantation, have greatly improved the outcomes of affected patients, this disease is still characterized by high relapse rates, with most patients eventually dying of lymphoma progression. Before official approval by EMA, patients with relapsed/refractory mantle cell lymphoma with unsatisfied critical medical urgency were granted ibrutinib early access through a Named Patient Program in Italy (NPP). An observational, non-interventional, retrospective, multicenter study focuses on collecting information about the effectiveness and safety of ibrutinib as single-agent in patients who received at least one dose of ibrutinib under the NPP in the period between 29/Jul/2014 and 25/Jan/2015 in Italy was conducted. Data from patients treated with ibrutinib outside a controlled clinical trial within a NPP could give additional information about the clinical use, treatment duration, efficacy and toxicity of ibrutinib given to relapsed or refractory MCL patients in a real life context. Sixty-six heavily pretreated patients were enrolled. They had received a median of previous therapies of 3, comprising lenalidomide, bortezomib, temsirolimus and autologous stem transplant. At the end of therapy there were 11 complete responses, 11 partial responses, 5 stable diseases and 37 progressions of disease leading to an overall response rate of 33.3%. At 3 years overall survival was 38.4% and disease free survival 75% at 2 years: 10/11 patients are in continuous complete response with a median of 15.5 months. Hematological toxicities were manageable, 8 thrombocytopenia occurred, of which only 2 grade 4 and the other 6 related to ibrutinib. Main extra-hematological toxicities were diarrhea (9.4%) and lung infections (9.0%) which all lead to early drug discontinuation. Overall, 4 atrial fibrillations and 3 hemorrhagic syndromes occurred. Our results are superimposable to those obtained in clinical trials: thrombocytopenia, diarrhea and lung infections are the relevant adverse events to be clinically focused on; for effectiveness, ibrutinib is confirmed to be a valid option for refractory/relapsed MCL also in a clinical setting mimicking the real world.

C098

REAL LIFE EXPERIENCE WITH BRENTUXIMAB VEDOTIN: THE ITALIAN STUDY ON 40 RELAPSED/REFRACTORY ANAPLASTIC LARGE CELL LYMPHOMA PATIENTS

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From November 2012 to July 2014, brentuximab vedotin (BV) was available in Italy for patients with relapsed systemic anaplastic large cell lymphoma (ALCL) based on the national law 648/96. A large Italian observational retrospective study was conducted on the use of BV in the everyday clinical practice to check if clinical trial results are confirmed even in a real life context. Primary endpoint was the best response; secondary endpoints were the overall response rate at the end of the treatment, duration of response, survival and the safety profile. A total of 40 ALCL (18 anaplastic lymphoma kinase [ALK] negative and 22 ALK-positive status) patients were enrolled. Patients were heavily pretreated (including autologous transplant in the 32.5% of cases). Best response was observed after a median of 4 cycles in 31 patients (77.5%): 19 (47.5%) patients obtained a complete response (CR) and 12 (30%) achieved a partial response (PR); overall response rate at the end of the treatment was 62.5% (18 CR and 7 PR). The best response rate was higher in the elderly subset (>60 years): 9 (64.2%) CR and 3 (21.4%) PR, achieving a total of 85.6%. At the latest follow up 15/18 patients are still in CR (3 with consolidative procedure). Global progression free survival was 39.1% at 29 months and disease free survival 54% at 23.9 months (median not reached). Median duration of response was 12 months (range 9-24 months). We identified 5 long term responders (patients with a response ≥ 12 months), all were still in CR at the latest follow up (1 underwent allogeneic transplant). Particularly, all the long term responders were aged <30 years at first BV infusion. The treatment was well tolerated even in this real life context and the toxicity profile was closely similar to the previously published data. Toxicity was primarily neurological and rarely so serious as to require treatment reduction or interruption. No long-term toxicity was assessed during the follow-up period, even in patients later subjected to transplant consolidation. BV induces clinical responses quite rapidly, permitting the timely application of the transplantation phase. Furthermore, BV displays a favorable toxicity profile, without overlapping toxicities with most of the agents employed in high-dose conditioning regimens. For patients ineligible for transplant or for who transplant failed, BV may

represent a feasible effective therapeutic option in everyday clinical practice.

C099

RITUXIMAB, BENDAMUSTINE AND CYTARABINE (R-BAC) IN PATIENTS WITH RELAPSED-REFRACTORY AGGRESSIVE B- AND T-CELL LYMPHOMA

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Relapsed or refractory (R/R) aggressive lymphomas (*i.e.* diffuse large B-cell lymphoma -DLBCL-) have poor outcome, especially if not candidate to consolidative autologous stem cell transplant (ASCT). No standard therapy exists. The combination of rituximab and bendamustine is associated to progression-free survival (PFS) of 3 to 8 months. In this pilot multicenter study we evaluated the safety and efficacy of rituximab, bendamustine and cytarabine (R–BAC), as salvage treatment in R/R aggressive B- and T-cell lymphomas not eligible to ASCT. Twenty-seven patients (16 DLBCL, 9 transformed [t-]DLBCL and 2 peripheral T-cell lymphoma), aged 37-84 years (median 68), were included and treated in four Hematology Institutions. R-BAC consisted of rituximab (R, 375 mg/m^2 intravenously [IV], day1), bendamustine (B, 70 mg/m^2 IV, days 2 and 3), and cytarabine (500 mg/m2, IV on days 2 to 4) every 21/28 days, up to 6 cycles. All patients had received anthracycline containing induction therapy (CHOP or CHOP-like), five (19%) had previous ASCT, and median number of previous treatment was 2 (range 1-4). Median time from initial lymphoma diagnosis was 29 months (4-120). Overall, 52% had relapsed disease, and 48% had refractory disease, with 15% of patients being refractory both to CHOP and DHAP/GDP. Response was assessed according to IWG 2007 criteria. Patients received a median of 4 cycles of R-BAC (2-6). Overall, OR was 74%, and CR was 48%. Among different histologies, OR was 75% in DLBCL (CR 50%), 78% in t-DLBCL (CR 56%), and 50% in T-cell lymphoma (one partial remission, one stable disease). Refractory patients had an OR of 62% (CR 30%). The median overall survival and PFS were 15.4 (9-19) and 10.2 (7-14) months, respectively (Figure 1). Median duration of response was 14.7 months (4-24). Treatment was well tolerated, with main toxicity being hematological, as expected. Treatment discontinuations before cycle 4 were due to toxicity/adverse events in 17%, progressive disease in 17%, and other reasons in 13%. Nine patients (39%) received cytarabine dose reduction (2 days instead of 3), due to advanced age or toxicity. Neither number of previous lines nor relapsed *vs* refractory disease were associated with significantly different PFS. R-BAC had promising activity with an acceptable toxicity profile in this small explorative cohort of heavily pretreated R/R aggressive lymphomas. Our results suggest that R-BAC should be further investigated in this setting.

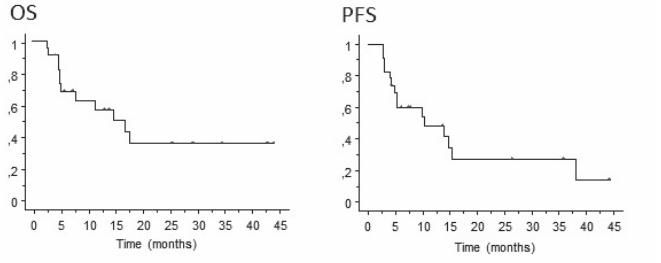

Figure 1.

C100

NEUTROPHIL/LYMPHOCYTE RATIO (N/L) AS A NEW PROGNOSTIC MARKER IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA: A PROSPECTIVE STUDY FROM RELLI

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The neutrophil/lymphocyte ratio (N/L) at diagnosis has been shown to be a prognostic factor for survival in solid tumors. An increase in the neutrophil count is a marker of inflammation which is an essential part of the neoplastic process. Conversely, a decrease of the peripheral lymphocyte count might reflect an impairment of the host defense mechanism associated with advanced and aggressive cancers. As there are only few reports on the N/L ratio in diffuse-large-B-cell lymphoma (DLBCL), we investigated the prognostic role of this score in 286 newly diagnosed patients, enrolled from 1 January 2013 to june 2016 in a multicenter prospective registry of the Lazio region in Italy. Median age at diagnosis was 69 years (27-91) and the female/male ratio was: 141/145. The associations between N/L ratio and patient characteristics was analyzed. The optimal cut-off value for the N/L was obtained using the Receiver Operating Curve (ROC) and according to the published data in solid tumor. 142/286 (49%) patients had a N/L ratio <4 and 144/286 (51%) patients a N/L ratio >4. N/L ≥4 was significantly associated with presence of B-symptoms (p=0.01) and elevated LDH levels (p=0.007) at diagnosis. 257/286 (90%) were treated with R-CHOP (rituximab, cyclophosphamide, Adriamycin, vincristine, and prednisone) or R-CHOP-like. Complete Remission (CR)+Partial Remission (PR) were obtained in 210/286 (73%). The median follow up period was 15 months (range: 1-33 months): 27 patients died for lymphoma relapse/progression and 16 for other causes. Patients with N/L ≥4 exprienced a higher rate of relapse, while N/L <4 was associated to a significantly better Overall and Event Free Survival (OS: P <0.05; EFS: P<0.01) (Figure 1).Furthermore, considering only patients with IPI score ≤3, those with N/L <4, (Figure 1), had a better OS compared to those with N/L ≥4 (P<0.01). *Conclusions:* The N/L ratio may be a useful and inexpensive prognostic marker in patients with DLBCL. The inferior outcome observed in patients with N/L ≥4 might reflect an immune and inflammatory imbalance induced by a more aggressive tumor, releasing directly or indirectly inflammatory cytokines and/or inducing immune suppression or exhaustion. A link with inflammation is suggested by the correlation of N/L ratio ≥4 with high LDH levels and the presence of B symptoms.

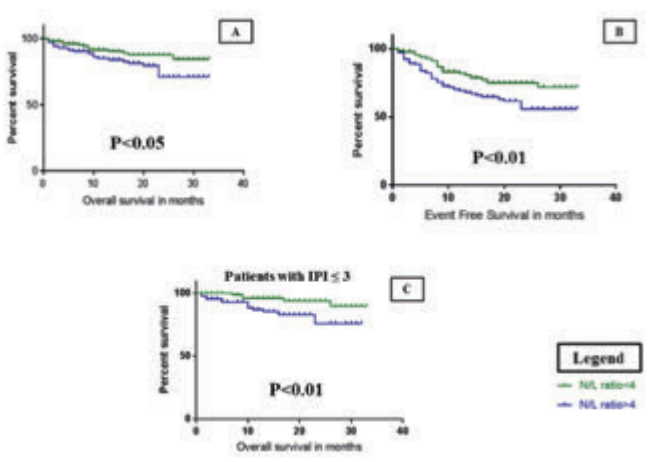

Figure 1.

POSTER

Cytogenetics and Laboratory Investigation

P001

ELEVATE LEVELS OF KAPPA FREE LIGHT CHAINS IN CSF SUPPORT THE DIAGNOSIS OF MULTIPLE SCLEROSIS CAN BE PREDICT CLINICALLY ISOLATED SYNDROME CIS-SM CONVERSION?

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Study: This study has shown an elevated concentration of kappa Free Light Chains (k FLCs) in cerebrospinal fluid (CSF) of multiple sclerosis (MS) patients and in patients with Clinically Isolated Syndrome Suggestive of MS (CISSMS). Our objective was to determine the extent to which k FLC levels in CSF correlate with the diagnosis of MS or Clinically Isolated Syndrome Suggestive of MS (CISSMS), compared to oligoclonal banding (OCB) and Link's index. *Methods:* CSF from 145 patients (including MS and CISSMS patients) and 10 negative controls was analysed using turbidimetry by SPA PLUS (Binding Site) and then, the results have been correlated with patients' diagnosis. *Results:* Mean value of k FLCs in the CSF of negative controls was 0,22 mg/L. This value has almost always exceeded in all patients with MS, whatever sex and kind of OCB. 0.22 mg/L could be used as basal value of every K FLCs dosage. MS patients show different values of k FLC, this underline more sensibility of k FLCs than Isoelectric focusing (IEF). In this study we show patients with Clinically Isolated Syndrome (CIS) diagnosis with high concentration of k FLCs (mean value 2,19 mg/L). It would be interesting to monitor this patients and to verify the predictive quality of the test to evaluate CIS-SM convertion.

P002

TCR GAMMA CLONALITY ASSESSED BY NGS DOES NOT HELP TO DISTINGUISH EOSINOPHILOIA IN EGPA FROM HES

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Hypereosinophilia-associated syndromes are a heterogeneous group of diseases characterized by elevated blood eosinophilia with organ damage. Eosinophilic Granulomatosis with Polyangiitis (EGPA) and Hypereosinophilic Syndrome (HES) present several overlapping clinical and laboratory features, making it challenging to correctly insert patients in restricted and well-defined categories. Objective: to detect T cell receptor gamma (TCRG) clonal rearrangements in EGPA and HES, comparing the frequency of distribution of the V and J region segments in 21 patients afferent to the hematology, rheumatology or pulmonology divisions. *Methods:* Inclusion criteria were: a persistent eosinophilic count ≥1.5x109/L and signs or symptoms of organ involvement. Sequence-based determination of the frequency distribution of TCRG Gene Rearrangements was performed using next-generation sequencing with the Illumina MiSeq (LymphoTrack TRG assay, Invivoscribe). *Results:* We included 21 patients (9 with EGPA and 12 with HES). Four EGPA patients were MPO-ANCA positive. We detected TCRG clonal rearrangements in 44% patients with EGPA and in 42% patients with HES. No association was observed between TCRG clonal rearrangements and ANCA status in EGPA patients. The following recurrent TCRG gene rearrangements were observed: V 10J P1 (5 cases) and V 4J 1/2 (4 cases) were observed in both EGPA and HES, whereas V 9J 1/2 (2 cases) and V 10J 1/2 (2 cases) were observed only in patients with HES. The presence of TCRG rearrangement was not different according to the symptoms (asthma, vasculitis, skin, heart, gut, lung involvement, splenomegaly). IL2, IL5, IL4, eosinophil cationic protein (ECP), absolute eosinophils were measured: IL5 and ECP were higher in the polyclonal than in the clonal cases (9±2.5 *vs* 1.7±0.9; p=0.021 and 121.8±61.5 *vs* 39.5±1.5; p=0.07). On the contrary, no difference was observed in the absolute eosinophil count. The presence/absence of TCRG clonality did not significantly impact on the response to treatment (immunosuppressive or interferon) and on PFS. *Conclusions:* Even if preliminary, this study reveals a similar T cell receptor gamma repertoire in EGPA and HES, with recurrent rearrangements, thus suggesting a possible antigen-driven inflammatory response underlying hypereosinophilia in both EGPA and HES. Interestingly, this study confirms our previous results showing the TCR delta rearrangement (assessed by qualitative PCR) in 40% of the EGPA patients.

P003

HASHCLONE: A NEW BIOINFORMATICS SUITE TO QUANTIFY MINIMAL RESIDUAL DISEASE BY NEXT-GENERATION SEQUENCING IN B-CELL LYMPHOMA

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Minimal residual disease (MRD) assessment by PCR approaches (ASO qPCR) has gained great importance in lymphoma response evaluation. Nevertheless, labor intesiveness, failure of marker identification and potential false-negative results affect PCR success. Next-generation sequencing (NGS) techniques applied to MRD studies showed good performances, overcoming intrinsic PCR limitations. However, they are strictly dependent by a computational analysis, that manages the huge volume of deep sequencing data. We present HashClone, a new bioinformatics tool that provides, from NGS data, marker assessment at diagnosis and MRD monitoring over time. HashClone was tested in the context of mantle cell lymphoma (MCL), in order to detect the major clone and monitor it over time. Five MCL patients, enrolled in a Fondazione Italiana Linfomi (FIL) prospective clinical trial, were studied for immunoglobulin heavy chain rearrangements (IGH) and MRD monitored by both ASO-qPCR and a BIOMED II amplicon-based, NGS approach.

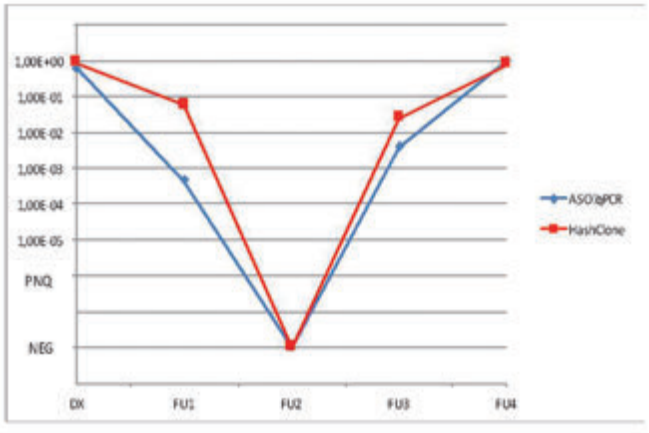

Figure 1.

For each patient, peripheral blood or bone marrow samples from diagnosis, three artificial follow-ups and samples collected during the clinical trial were analysed. HashClone pipeline, firstly, defined a set of putative clones; then, IGH was classified by the link to IMGT database (http://imgt.org). Finally, HashClone MRD results were compared to data previously obtained by ASO qPCR. HashClone was tested on 39 MCL samples. For the five diagnostic samples, HashClone identified

an average value of 1008 putative clones. Of these, only 21, having a frequency higher than 5%, were considered MCL related clones by IMGT filtering. Each diagnostic sample displayed only one predominant clone, with a median frequency of 98%. Compared to Sanger sequencing data, all the major clones showed the same IGH with a 100% of nucleotide homology in 4 out of 5 cases. Correlation analysis showed a high concordance between ASO qPCR and NGS (r2=0.85). Finally, superimposable performances were observed in single patients MRD monitoring compared to ASO qPCR results (Fig1). HashClone is a new bioinformatics tool for the identification of IGH clonality in MCL patients. It is not affected by biologic biases and is the first tool able to extrapolate the temporal evolution at several time points ("MRD monitoring"). Since this is the first HashClone application, the tool needs to be fine-tuned and validated on large samples series, before being used it in large and prospective clinical trials.

P004

RARE AND UNUSUAL CALR AND MPL GENES MUTATIONS IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS: SINGLE CENTRE EXPERIENCE

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Driver mutations in Philadelphia-negative myeloproliferative neoplasms (MPNs) are often mutually exclusive and include JAK2, CALR and MPL. JAK2V617F is present in 90-95% patients with Polycythemia vera (PV) and in 50–60% of those with Essential thrombocythemia (ET) or Primary myelofibrosis (PMF). CALR and MPL mutations are absent in PV and their frequencies are 20–25% and 3–4%, respectively, in ET, and 20–25% and 6–7% in PMF. It is known that there are 36 types of somatic mutations in CALR (insertions and deletions) but the most frequent are that of type I (52-bp deletion) and type II (5-bp insertion); about MPL the most common mutation results in an aminoacid substitution (either lysine or leucine) at the 515 position (MPL W515K or MPL515L). The aim of our study was to evaluate CALR and MPL gene mutations in JAK2 negative MPNs patients to find unusual and rare mutations of these two genes. Samples of 156 patients (M/F: 62/94) with documented JAK2 negative MPNs, observed at our center between 2005 and 2016, were re-evaluated for CALR and MPL mutations. Mutations in MPL (exon 10) gene and CALR (exon 9) gene were investigated by sequencing analysis. CALR mutation was found in 51 patients (32.7%): 27 (52.9%) and 19 patients (37.3%) showed type I and type II mutation respectively; 5 patients (9.8%) showed the following patterns: del p.K375 fs*49, delins p.384fs*49 complex, delins p.D384fs*46 complex, Pk377fs*55 complex, p.Glu369fs*38. Six patients (4%) were positive for MPL mutations: 4 patients (66.7%) showed the common mutation W515 (3 patients: W515L, 1 patient: W515K), 2 patients (33.3%) showed rare mutations: W515-P518delinsKT and W515A. Few literature reports are available about the possible prognostic significance of variant mutation of MPL and CALR genes. Our efforts are now oriented to collect samples from a larger cohort of patients to undestand the impact of these rare and uncommon patterns of mutations on clinical outcome of patients with MPNs in particular way in the era of new drugs.

P005

THE ASSESSMENT OF SOMATIC MUTATIONS IN ACUTE MYELOID LEUKEMIA IN 2017: A PREDICTIVE MODEL

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After the recent WHO revision, AML patients are now classified as at low, intermediate or high risk on the basis of cytogenetic and molecular integrated parameters. A web available algorithm where clinical, cytogenetic, and molecular features are integrated is now available at http://cancer.sanger.ac.uk/aml-multistage/. The NGS techniques today allow an optimal characterization of each patient; nevertheless, the optimization of NGS is often complicated and it is not available everywhere. Thus, in small laboratories, conventional PCR is still an useful technique for the mutational assessment. In this study, we used the Qiagen AML real-time plates for assessing ASXL1, TET2, IDH1, IDH2, NRAS, WT1, c-KIT, RUNX1, DNMT3A mutations in addition to the conventional diagnostics tests in 38 AML patients at diagnosis. Their median age was 59 years, and 22 were male; in 7 cases AML followed a MDS and in other 7 a previous chemotherapy. Half of patients presented an intermediate, and 1/3 an unfavorable karyotype. After induction, 60% of patients achieved a complete or partial response; the 3-years OS was 31% and PFS 27%. In univariate analysis, the quality of response and karyotype influenced the OS; in addition, the PFS was influenced also by the FLT3 mutation and the allogeneic transplant performance. In multivariate analysis, only the quality of response retained its prognostic value. About somatic mutations, 16% of patients was mutated for NPM1, 13% for FLT3, 24% for N-RAS, 8% for WT1, 10% for IDH1, 16% for IDH2, 50% for c-KIT, 10% for RUNX1, 3% for ASXL1, and 8% for DNMT3A. Thus, 30 out the 38 enrolled patients presented at least one mutation (30%, 3 mutations); after the adjunctive analysis, 18/38 cases (47%) translated to a poorer risk category, even if no significant impact of the adjunctive mutations on OS and PFS was observed. This was an observational study, thus our therapeutic strategy did not change; nevertheless, we inserted the mutational data in the web site to calculate the probability of survival at 2 years. Then, we compared the output with the real outcome: a discordance of 13% between the prognostic model and the real outcome was observed when we used all the mutational data, but it increased to 23% when only routine data were used (translocations, NPM1, FLT3). In conclusion, the basic molecular assays are fundamental for AML prognostication, but also adjunctive somatic mutational assessments would be useful in the clinical practice.

P006

A NEW IMPROVED DIASORIN Q-LAMP ASSAY FOR THE OPTIMAL AND ULTRA-FAST DETECTION OF BCR-ABL1 COMMON AND RARE ISOFORMS

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Introduction: The molecular detection of BCR-ABL1 transcripts is required to confirm the diagnosis of Philadelphia Positive Leukemias at onset. Although RT-PCR is the most diffuse molecular method so far, a faster and reliable Q-LAMP based assay is nowadays entering in laboratory routine. The Q-LAMP BCR-ABL1 assay detects and discriminates in one hour the most common isoforms of BCR-ABL starting directly from RNA in a one-step, close tube format. In this study, we evaluated a new enhanced formulation designed to detect also less frequent isoforms of p190 and p210 (e1a3, e13a3/b2a3, e14a3/b3a3) and the p230 transcripts (e19a2, e19a3). *Methods:* The new Q-LAMP BCR-ABL1 consists in a fluorescent multiplex assay for differential detection of both p190 and p210 BCR-ABL1 (common and rare transcripts) and GUSB endogenous RNA, which acts as internal control. Patient RNA is retro-transcribed and amplified at constant temperature for 60 minutes on the Liaison IAM instrument that displays fluorescent signals in real time and return final elaborated data. The assay has been tested on a total of 148 clinical samples: 52 BCR-ABL1 negative and 96 BCR-ABL1 positive (p190 n=38 and p210 n=56), among which 15 samples presented uncommon isoforms (e1a3 n=5; b2a3 n=8, b3a3 n=2, e19a2 n=2). Results were compared with the ones obtained by conventional RT-PCR method (Biomed Protocol). *Results:* the new enhanced BCR-ABL1 Q-LAMP assay showed 100% concordance with RT-PCR, with average amplification time of 23,41 minutes for common isoforms and 31,3 minutes for rare isoforms. In particular p210 common isoforms average amplification time is 21,16 min respect to 25,03 min of the rarer p210 isoforms and p190 common isoforms average amplification time

is 26,54 min respect to 36,84 min of the rarer p190 isoforms. All negative samples presented the exclusive amplification of the internal control GUSB RNA. *Conclusions:* the enhanced BCR-ABL1 Q-LAMP assay well demonstrated to be able to detect both common and uncommon isoforms of p210 and p190, as well as the rare isoform p230. This new feature, combined with the speed, the robustness and the safety of the one-step, close tube format, allow laboratories to decrease the risk associated to the multistep RT-PCR procedure, saving time and optimizing workflow. In conclusion, the Q-LAMP BCR-ABL1 in this new formulation represents a convenient and reliable tool for molecular diagnosis of Philadelphia Positive Leukemias, enhancing patients management.

P007

IN THE CHRONIC MYELOID LEUKEMIA THE SALIVARY PROTEOMICS RECAPITULATES SOME ASPECTS OF THE LEUKEMIC STEM CELL AND ITS NICHE

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It has been reported that the salivary proteomics could be representative of the behavior of many diseases. Our group previously reported that the re-appearance of immunoglobulins in the saliva represented a predictive marker of relapse in indolent lymphomas. In this study, we investigated the saliva proteomic profile in 2 groups of patients with chronic myeloid leukemia (CML), the first one represented by a pool of 4 patients in stable deep molecular response during imatinib treatment (undetectable 4.5 MR), and the second one by a pool of 4 sex and age-matched failed cases. The proteomics analysis was performed on albumin and IgG depleted saliva samples through a nanoLC-ESI-MSMS with SWATHTM acquisition method. Overall, 30 proteins resulted down-regulated and 34 up-regulated in resistant *versus* sensitive cases. Among the proteins down-regulated in resistant cases, of interest are: myeloperoxidase and thymosine beta4, that control the myeloid differentiation; MMP-9, involved in the reduction of fibrosis that is a fundamental mechanism for preserving the LSC in the bone marrow niche; peroxiredoxin-2, related to the osteoblastic differentiation (of note that osteoblasts are one of the principal components of the endosteal niche where LSC hides); catalase, that, after activation of the BCR-ABL1/STAT5 pathway, is responsible for the LSC quiescence. Among the up-regulated proteins in resistant cases, we observed: SPARC-like protein 1, that is a matrix-linked protein; transgelin-2, involved in IL8 level increase (IL8 sustains the angiogenesis and increases the adhesion of the LSC to the stroma); leucocyte elastase inhibitor, that inhibits the myeloid differentiation; carbonic anhydrase-6, that contributes to make hypoxic the niche and to stimulate myeloid suppressor cells (it is known that hypoxia in the niche is responsible for the loss of the BCR-ABL1 protein, with the consequent resistance to TKIs); kallikrein-1 and -11, that are implicated in the carcinogenesis in several solid tumors. These are preliminary results, but it's really surprising that some proteins that are niche-related are present in the saliva of CML patients. This is particular relevant considering that the niche seems to play a fundamental role in LSC survival. Thus, the saliva proteomics profile could contribute to the identification of patients that are really good candidates for the TKI discontinuation.

P008

BIOLOGICAL AND CLINICAL SIGNIFICANCE OF EXTRACELLULAR VESICLES IN HEMATOLOGICAL MALIGNANCIES

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Extracellular vesicles (EVs) are lipid bilayer particles (30-2000nm in diameter) released by normal and neoplastic cells. According to their size, EVs can be divided in exosomes, microvesicles and apoptotic bodies and are enriched in protein, mRNA, miRNA and DNA. EVs mediate intercellular communication interacting with target cells and controlling fundamental biological functions. In this setting, we first demonstrated that EVs mediate communication between umbilical cord blood CD34+ (UCB-CD34+) and bone marrow mesenchymal stem cells (BM-MSCs). The exposure of UCB-CD34+ to BM-MSC EVs influenced UCB-CD34+ cell fate modifying their gene expression profile and rendering them more viable and less differentiated. Moreover, BM-MSC EVs treatment in *in vivo* mouse model caused an augmented migration of CD34+ from peripheral blood to BM niche indicating that BM-MSC-EVs could be helpful in BM microenvironment reconstitution in transplant applications. EVs are present in different biological fluids, including serum, and have a potential role as disease biomarkers. We observed that EVs levels are significantly elevated in serum of patients affected by different hematological malignancies (HMs), such as chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML) and Waldenstrom macroglobulinemia, respect to healthy controls. Furthermore, EVs from patients specifically express tumor-related antigens and correlate with different HM clinical parameters. In particular, in CLL we found that absolute number of B-cell derived EVs significantly correlate with high tumor burden. Moreover, absolute MV number cutoff selected by ROC analysis distinguished Rai stage 0 patients with shorter time to treatment (TTT) from those with more stable disease. Likewise, in the entire cohort, 2 groups of patients with different overall survival (OS) and different TTT were identified. At multivariate analysis, serum EVs independently predicted for OS (along with Rai stage) and TTT (along with Rai stage, lymphocytes, CD38). In addition, analyzing their content, we found that the EV miR155 levels are significantly higher in different HM compared to controls. EV miR155 ROC curve analysis reveal significantly different patterns in CLL and AML compared to controls and in AML compared to myelodysplastic syndromes. In conclusion, our data indicate that circulating EVs and EV miR155 could represent new attractive biomarkers in HMs

P009

COMPARISON OF BONE MARROW ASPIRATE FLOW CYTOMETRY AND BONE MARROW HISTOPATOLOGY IN PATIENTS WITH MANTLE CELL LYMPHOMA: EVALUATION OF CONCORDANCE

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Background: Bone marrow (BM) biopsies is routinely performed in patients affected by Mantle cell lymphoma (MCL) at the diagnosis and it may implicate different therapeutical approach. In our retrospective study we decided to investigate the utility of multiparameter flow cytometric (FC) examination of bone marrow aspirates at the diagnosis and to evaluate the concordance rate with BM biopsies evaluation. *Methods:* In our center we collected 80 new diagnosis of mantle cell lymphoma from January 2010 to February 2017. Fourty-three patients were retrospectively evaluable for the presence of concomitant BM biopsy and aspirate at the diagnosis. Seventeen patients did not performed BM examination at diagnosis for age. Twenty patients underwent histopathological evaluation at diagnosis without FC analysis. We used 4-tubes flow cytometry for FC analysis with the following fluorochrom conjugated monoclonal antibodies: CD5, CD10, CD11c, CD19, CD20, CD23, CD25 CD45, CD79B, FMC7, CD200, and Ig kappagamma light chain. *Results:* Among these 43 patients, 8 patients had a diagnosis of MCL based on BM histomorphology, 35 based on lymph node evaluation. BM biopsy was positive in 30 cases, negative in 13 cases. The concordance rate between histomorphology and flow cytometry was 81.4% (n=35). Six cases (14%) were detected by flow cytometry alone and missed by histomorphology analysis. We examined the status of bcl-1 detected by RT-PCR at the diagnosis and it was positive in 3 of these cases; RT-PCR was not performed in the remaining 3 cases. We repeated the test after therapy and both samples were negative in all of these six cases. Two cases (4,7%) were histopathologically positive and immunophenotypically negative; in both cases bcl-1 was negative.

Four patients (9,3%) performed flow cytometric examination of peripheral blood only at the diagnosis and every cases coincide to BM histomorphology. *Conclusions:* At present, lymph node biopsy is the standard for MCL diagnosis. Based on these evidences, multi-color flow cytometry can be an useful method for assessing bone marrow infiltration and plays a complementary role to histomorphology. It facilities a rapid and accurate diagnosis of mantle cell lymphoma expecially in patients with no lymph node involvement. Futhermore, it is a less invasive test and could be performed also in elderly patients for a correct staging and treatment. *Acknowledgment:* This study was supported by Legato Zottola Donation and AIL Pistoia

Anemias and Myelodysplastic Syndromes

P010

REFRACTORY ANEMIA WITH RING SIDEROBLASTS (RARS): ASSESSMENT OF MITOCHONDRIAL DNA MUTATIONS

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of bone marrow disorders characterized by ineffective hematopoiesis with cytopenias and risk of developing acute myeloid leukemia (AML). RARS is a subtype of MDS characterized by isolated erythroid dysplasia with 15% or more of the erythroblasts in the bone marrow containing iron-laden mitochondria forming a ring around the nucleus. *Aims:* The aim of the present study is to assess the presence and significance of any alterations of the mitochondrial genome in the pathogenesis of RARS. We evaluated a total of five patients with RARS diagnosis. *Methods:* After clinical diagnosis, Perl's staining, karyotyping and MLPA assay were performed on bone marrow. Total DNA was extracted from CD34, bone marrow, peripheral blood and buccal brushing cells and mitochondrial DNA (mtDNA) was Sanger sequenced. *Results:* Several mtDNA mutations were found in all patients but particularly in one, a 56 years old man, who early progressed in RAEB2/AML. We found homo- and heteroplasmic mutations at different proportions in the analyzed cell types. *Conclusions:* A possible role of the mtDNA mutations including the novel ones which might be specific of CD34 cell population will be discussed as possible contributing factor to the disease to establish whether mtDNA might represent a novel molecular marker for the diagnosis and/or prognosis of RARS. *Acknowledgement:* This research was supported by "Associazione italiana contro le leucemie-linfomi e mieloma, Sezione di Matera (MATERAIL)"

P011

PREVALENCE OF HFEH63D MUTATION IN PATIENTS WITH IDIOPATHIC ERYTHROCYTOSIS

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Background: Idiopathic erythrocytosis (IE), characterized by persistently raised hemoglobin (Hb) and hematocrit (Ht) levels, has not a definite cause, even after an accurate diagnostic investigation. Increased levels of ferritin are often observed in IE and we recently found that mutations or polymorphisms of HFE gene are commonly found in patients with IE. Curiously, HFE mutations were found also in IE patients with normal ferritin levels. To better understand the relation between erythrocytosis and HFE gene, we searched which mutations were prevalent in IE patients. *Materials and Methods:* HFE mutations were searched in 33 patients with IE (30 males and 3 females, mean age 53±13 y, Hb 175,5±9,2g/dL, Ht 51,2±3,0%) not carrying JAK2V617F or JAK2 exon 12 nor EPOR, VHL, PHD2 or HIF-1 alpha genes mutations. All the patients had normal serum erythropoietin level. None had an overt clinical diagnosis of hemochromatosis. HFE gene mutations was performed using Lightcycler 480 on extracted DNA. *Results:* We found 14 HFE mutated patients (42%): 10 heterozygous and 2 homozygous H63D and 1 H63D/C282Y. Only 1 patient carried heterozygous S65C mutation. On a whole, 13 (93%) of the HFE mutated patients have at least one H63D mutated allele. In the Table we report the frequencies of HFE mutations, H63D mutations and high ferritin between mutated patients in general population, in IE patients and in the present cohort. *Discussion:* We have recently observed that increased serum ferritin levels and mutations of HFE are more common in IE patients than in the European general population. The present study shows that most of the HFE mutated patients with IE carry at least one H63D allele. HFEH63D polymorphism, which is rarely associated with iron overload but drives an impairment in iron metabolism, may be linked with erythrocytosis in spite of ferritin levels. Then, it remains unclear how HFE mutations alter the supply of iron to the erythroid tissues. Study of a

large cohort of IE patients are needed to confirm our results and to clarify the physio-pathological relation between HFE or other molecules involved in iron metabolism and erythrocytosis.

Table 1. Frequencies of HFE mutations, H63D mutations and high ferritin between mutated patients in general population, in IE patients and in the present cohort.

	Italian General population	IE* 56 patients	IE in the present study 33 patients
HFE mutated	26.5 %	25 (44.6%)	14 (42%)
*HFE*H63D	23%	17 (30.4%)	13 (39%)
High ferritin levels in mutated patients	15%	12 (48%)	9 (64%)

* Biagetti G, Catherwood M, Robson N, Bertozzi I, Cosi E, McMullin MF, Randi ML (2017). HFE mutations in idiopathic erythrocytosis. *British Journal of Haematology*, DOI: 10.1111/bjh.14555.

P012

AZACYTIDINE TREATMENT IN ELDERLY MYELODYSPLASTIC PATIENTS MAY INDUCE LONG-LASTING REMISSION ALLOWING SOMETIMES ITS DISCONTINUATION

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Azacytidine (AZA) is a subcutaneous hypomethylating agent approved for treatment of elderly patients (pts) with Intermediate-2/High-risk Myelodysplastic Syndrome (MDS) according to the international prognostic scoring system (IPSS) and chronic myelomonocytic leukemia (CMML) who are not eligible for hematopoietic stem cell transplantation (HSCT). AZA treatment is continued as long as the patient is responsive. We evaluated 34 pts treated with AZA (19 male/15 female, median age of 73 years, range 51-91) with the following diagnosis according to 2016 WHO classification: 1 MDS patient with single lineage dysplasia, 1 MDS patient with multi lineage dysplasia, 11 MDS pts (32%) with excess blasts–1, 19 MDS pts (55%) with excess blasts–2 and 2 pts (6%) CMML. All pts performed at least 1 course of AZA treatment with a median number of 13 courses (range 1-60). According to International Working Group, clinical response was evaluated in 21 patients who received at least 6 courses of treatment with the following responses: 3 complete remission (CR) (14%), 1 partial response (PR) (5%), 6 stable disease (29%), 5 progression disease (24%), 7 Acute Myeloid Leukemia (AML) transformation (33%). Noteworthy, the MDS patient who achieved PR during AZA showed a del(7q) at diagnosis, no longer detected after 6 course of treatment. AZA treatment also decreased transfusion requirement in 6 of 9 patients with transfusion blood dependence who receiving at least 6 courses of treatment: 4 pts achieved transfusion independence while 2 decreased transfusion requirement. In our cohort, AZA discontinuation of treatment was due to AML transformation (26%), loss of response (12%), toxicity (increased transfusion requirements) (6%), HSCT (6%), CR (6%), death (24%). Noteworthy, two MDS patients within the second year of AZA treatment achieved CR, transfusion independence and Erythropoietin responsiveness, allowing AZA discontinuation still ongoing after 52 and 34 months follow-up. The median overall survival was 23 months, with a rate of pts alive and in treatment after 1 /2/3 years of 56% (19 pts), 30% (10 pts) and 9% (3 pts), respectively. Our data provides further evidence that AZA may be effective in elderly patients with MDS and CMML, improving quality of life, decreasing blood transfusion dependence and occasionally inducing long-lasting CR.

P013

SUCROSOMIAL IRON *VS* DIFFERENT IRON ORAL FORMULATION IN IRON DEFICIENCY ANEMIA DUE TO GASTROINTESTINAL BLEEDING: MULTICENTRIC RANDOMIZED STUDY

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Background: Ther'are several different oral iron formulation with different mechanisms of uptake known or supposed (DMT1 for iron sulphate, microencapsulated iron and sunactive iron, transcellular and lymphatic way for sucrosomial iron, heme and peptones carrier for heminic chelated bisglycinated iron, peptones carrier for chelated bisglycinated iron). *Aim:* Data regarding absorbtion and effectiveness for each kind of iron are lacking. Aim of this study is to see if ther'is some difference regarding effectiveness and tolerability among different oral iron formulation. *Methods:* This study is a multicentric randomized study. 300 patients with iron deficiency anemia in gastrointestinal bleeding were randomized 1:1:1:1:1:1 to receive iron sulphate (65 mg of elemental iron oid), microencapsulated iron, sunactive iron, sucrosomial iron, heminic chelated bisglycinated iron (30 mg of elemental iron tid), chelated bisglycinated iron (15 mg of elemental iron tid). Patients charachteristics were similar in all six groups. Hemoglobin trend and side effects were recorded in general patients population and in C reactive protein (CRP) high level patients. Median Hb value at start of treatment was 8.2 g/dl. In group of patients with high CRP median Hb value was 7.8 g/dl. Median follow-up was 4.5 months (R 3-6). *Results:* The Hb increase rate in the first two weeks of treatment is the same in the group of sucrosomial, chelated bisglycinated and heminic bisglycinated iron, but from the third to the sixth week Hb increase rate is higher in sucrosomial iron group. In this group from the sixth to the twelfth week the Hb increase rate is still higher, but shows a slight decrease. In all group Hb level achieves a plateau phase after three months and ferritin level starts to increase. At three monts higher levels of hemoglobin are present in sucrosomial iron (13.2 g/dl), heminic chelated bisglycinated iron (11.7 g/dl), chelated bisglycinated iron (11.3 g/dl). In group of patients with high CRP level (>30 ng/ml) the Hb increase is higher in sucrosomial iron group from the tenth week, is continuous until the sixth month (Hb 12.5 g/dl) and is linked to a marked decrease of CRP (5 ng/ml). All type and grade of side effects are higher in ferrous sulphate group (15/50) and in sunactive iron group (6/60). *Conclusions:* Among different oral iron formulation in iron deficiency anemia sucrosomial iron shows a faster activity, an higher efficacy more evident in patients with high CRP value linked to a marked CRP level decrease after three month of treatment.

P014

ORAL HIGH DOSE SUCROSOMIAL IRON SUPPORT IS SAFE, FAST, WELL TOLERATED AND COST-EFFECTIVE AS INTRAVENOUS IRON IN SIDEROPENIC ANEMIA. MULTICENTRIC RANDOMIZED STUDY ON 200 PATIENTS.

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Background: In iron deficiency anemia support with intravenous iron allows a faster anaemia correction and a faster ferritin increase than iron sulfate. Frequently iron sulfate and intravenous iron generate adverse events as hypotension, urticarioyd reactions, shock, epygastralgia, constipation or diarrhea. High doses of oral iron frequently are poorly tolerated because of adverse events. *Aim:* Aim of this study is to verify if high doses of oral liposomial iron are safe, cost-effective and well tolerated as standard doses of intravenous ferrigluconate in patients with iron deficiency anemia. *Patients and methods:* We considered two group of patients (RANDOMIZED 1:1) with iron deficiency anemia due only to bleeding without other relevant comorbidities. In group A M/F was 1/1, 38 patients had haemorragic gastritis, 12 hemorragic enteric bleeding angiodysplasia, 50 hypermenorrhaea, median level of hemoglobin (Hb) was 8 g/dl (R 7.5-10.5), median ferritin level was 8 ng/ml (R 3-21), with a normal level of CRP or ESR, and received liposomial iron 30 mg 4 tablet/day.In group B M/F was 1/2, 50 patients had haemorragic gastritis, 4hemorragic enteric bleeding angiodysplasia, 46 hypermenorrhaea,median level of Hb was 8.2 g/dl (R 8-10), median ferritin level was 5 ng/ml (R 2-18), with a normal level of CRP or ESR, and received iv sodium ferrigluconate 62.5 mg iv in NS 100 ml in 3 h/day.The median treatment costs in each group were calculated considering the monthly global treatment cost for each patients in the treatment period. This

provided an estimate of the costs, independent of the precise cost of the drug, but tied to the final outcome (efficacy) of the therapeutic strategy used during the observation period. *Results:* In group A, 1 g Hb increase was observed after a median of 8 days (R 8-12), a targetHb level of 12 g/dl was achieved in a median time of 4 weeks (R 2-4) with a median cost of €110/months (R 92-162), 28 (28%) patients showed adverse events (16 epigastralgia, 12 diarrhoea). In group B, 1 g Hb increase was observed after a median of8 days (R 6-10), a target Hb level of 12 g/dl was achieved in a median time of 3.5 weeks (R 1.5-4) with a median cost of €326/months (R 250-360), 21 (21%) patients showed adverse events (8 hypotension, 13urticaria and headhace). *Conclusions:* Oral high dose liposomial iron support is safe, fast, well tolerated and cost-effective as intravenous iron in sideropenic anemia. This study needs confirmation on a larger cohort of patients.

P015

IRON CHELATION THERAPY IN TRANSFUSION-DEPENDENT MYELODYSPLASTIC SYNDROMES. RETROSPECTIVE STUDY OF 53 PATIENTS IN A SINGLE INSTITUTION

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Introduction: Several barriers may limit the initiation or the continuance of iron chelation therapy (ICT) in myelodysplastic syndromes (MDS): older age, comorbidities, poor tolerance and compliance. Therefore, with the aim of assessing the safety and efficacy of ICT in the daily clinical practice, we retrospectively analyzed our single-center experience on ICT in MDS patients with transfusional iron overload. *Methods:* From October 1997, in our Institution, 53 MDS pts (43 males), median age: 75 (36-96) yrs, with transfusion-dependent anemia, received ICT because of a diagnosis of iron overload. *Results:* 40 pts (75.5%) were affected by lower-risk MDS (IPSS risk: low or intermediate-1), while 13pts (24.5%) showed a higher-risk MDS (IPSS risk: high or intermediate-2). 33pts (62.3%) received deferasirox (DFX) as first-line treatment, 11 pts (20.7%) received DFX after a previous treatment with deferoxamine (DFO), while 7pts (13.2%) received DFO and 2pts (3.8%) received DFO after DFX because of contraindications to DFX or toxicity. Median time from diagnosis of MDS to the start of ICT: 20.5 months. Median number of RBC transfusions before the start of ICT: 40. Median serum ferritin (SF) level pre-ICT: 2111ng/ml; median SF after ICT (last value): 1954ng/ml; median duration of ICT: 12 (range 1-230) months. 7pts (13.2%) achieved a SF value <1.000, and 34 pts (64.1%) a SF value <2.500. Adverse events possibly related to DFX were observed in 27 cases (50.9%): renal: 12pts (22.6%) (grade >2: 1pt: 1.9%); gastrointestinal: 13pts (24.5%) (grade >2: 1pt: 1.9%); cutaneous: 2pts (3.8%) (grade >2: no pts). Permanent discontinuation of ICT: 26pts (49.1%), because of toxicity (13pts: 24.5%), worsening of clinical condition (4pts: 7.5%), discontinuation of transfusions (5pts: 9.4%), allogeneic transplantation (4pts: 7.5%). 4pts (7.5%) (with DFX: 3pts; with DFO: 1 pt) showed an erythroid response following ICT, after 2, 7, 32 and 112 months, respectively. 28pts (52.8%) died, because of infection (6pts), AML (4pts), cachexia (4pts), other neoplastic diseases (3pts), heart failure (2pts), and other causes (9pts). With a median follow-up of 24 (2-230) months, median overall survival (OS) was 51 months for all pts, 87 months for lower-risk pts and 24 months for higher-risk pts. *Conclusions:* In conclusion, in our experience ICT appears feasible and effective even in a population of elderly MDS pts, if carefully selected.

P016

CHARACTERIZATION OF PERIPHERAL BLOOD CIRCULATING EXOSOMES IN MYELODYSPLASTIC SYNDROMES

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Myelodysplastic syndromes are a heterogeneous group of clonal haematological malignancies affecting mainly the elderly and characterized by aberrant haematopoiesis, peripheral blood cytopenias and tendency to acute leukemia transformation. Exosomes are small vesicles of 100 nm diameter; they can be released by all cell types, and carry nucleic acids and proteins whose variation can represent a marker of disease initiation and progression. In our work, we isolated circulating exosomes from peripheral blood of myelodysplastic syndromes patients and from healthy donors evaluating the expression of 15 miRNA which have been demonstrated to be dysregulated during ageing and in inflammatory conditions. Exosomes were isolated from the serum of patients by differential ultracentrifugation steps performed on a sucrose gradient. Electron microscopy and nanoparticle tracking analysis demonstrated the purity and a good quality of exosome preparation. In addition, western blot and flow cytometry analysis showed that all samples were positively stained for tetraspanin CD 63. Among 15 miRNA studied. miR-9, miR-19b, miR-29a, miR-146a, miR-155, miR-39, miR-34a, miR-152, and miR-335 were not detectable both in patients and donors. On the contrary, mirRNAs 181a, 20a, 21, 17, 22 and were expressed, but only miR-126 showed a statistically significant downregulation in MDS patients respect to donors. Altogether these data suggest that peripheral blood exosomes could represent a valid source for the study of MDS markers, however further studies and standardization of techniques are required to optimize and develops new diagnostic tools.

P017

RESISTANCE TO AZACITIDINE IS DETERMINED AT CELLULAR LEVEL BY LOWER EXPRESSION OF NUCLEOSIDE METABOLIZING ENZYMES

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Azacitidine is at present the standard treatment for high risk MDS. We demonstrated that MDS patients responsive to azacitidine have significantly higher intracellular expression of the azacitidine-activating enzyme uridine-cytidine kinase-1 (UCK1) in bone marrow mononuclear cells (Valencia et al, Leukemia 2014). The possible role of azacitidine metabolizing enzymes in determining resistance to azacitidine has to be ascertained. *Objectives:* To confirm that cellular expression of nucleoside metabolizing enzymes plays a major role in resistance and significantly impacts on clinical response to azacitidine. *Methods:* Two cell lines, SKM1 sensitive (SKM1-S) and SKM1 resistant (SKM1-R) to azacitidine were analyzed for expression of UCK1, UCK2, hENT1, hCNT3, RRM1 and RRM2 by RT-PCR, and proteins were quantitated by western blotting. The expression of UCK1 and UCK2 was blunted by siRNAs (OriGene Technologies, MD, USA) in SKM1-S cells to determine their role in *in vitro* sensitivity to azacitidine. After transfection, cells were treated for further 48h with azacitidine at the concentrations of 0,1 and 1 M; apoptosis were evaluated by Annexin V test and the percentage of 5-methylcytosine was quantitated (Global DNA Methylation LINE-1 kit ActiveMotif, CA, USA). The expression of nucleoside metabolizing enzymes was evaluated prospectively in 18 IPSS high risk MDS patients treated with azacitidine. *Results:* SKM1-R cells did not express UCK1, UCK2, hENT1, hCNT3, RRM1 and RRM2. Corresponding proteins were not expressed. A reduction of apoptosis was observed in UCK1-silenced SKM-1 S after azacitidine 0.1 M treatment: non-silenced cells showed an increase of Annexin V-positive cells of 19% *versus* an increase of 3,4% in UCK1 silenced SKM-1; in UCK2- silenced SKM-1, after 0,1 uM AZA treatment we observed an increase of 9% of Annexine-V positive cells *versus* an increase of 18% in non-silenced cells. Hypomethylation induced by *in vitro* azacitidine treatment was also hampered by reduction of expression of UCK1 and UCK2. Gene expression of UCK1, UCK2, hENT1, hCNT3, RRM1 and RRM2 in primary cells did not predict different clinical response to azacitidine. We demonstrated that UCK1, UCK2, hENT1, hCNT3, RRM1 and RRM2 and the corresponding proteins are absent in azacitidine-resistant cell line SKM1-R suggesting to be the determinant of the induced resistance to azacitidine. UCK1 and UCK2 silencing induced by synthetic siRNAs significantly decreased azacitidine effects.

P018

EVALUATION OF PROGNOSTIC UTILITY OF WT1 EXPRESSION IN MIELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of hematological disorders, with differences in: number of hematopoietic lineages involved, need of supportive care, response to therapy (erythropoietin and/or hypomethylating agents), evolution in acute myeloid leukemia (AML). Among different prognostic scoring systems, R-IPSS (Revised International prognostic scoring system) based on levels of hemoglobin, Absolute Neutrophil Count, platelet count, bone marrow blasts and cytogenetic abnormalities is still the most used prognostic tool. WT1 gene is overexpressed in different types of solid tumors and AML. WT1 is a useful marker to monitor response to therapy and minimal residual disease. *Aim:* To evaluate if there is a correlation between WT1 levels at diagnosis and R-IPSS score and if WT1 could have a prognostic value concerning the risk of leukemia evolution in MDS patients. *Patients and methods:* We analyzed 33 patients with MDS. We evaluated WT1 expression by Real Time PCR. WT1 expression was normalized to the expression of the housekeeping gene Abl, using the following calculation: WT1/ABL *104. We correlated WT1 levels to the number of BM CD34+ cells, R-IPSS score and evolution to leukemia. *Results:* According to our preliminary data, WT1 expression seems to be lower in patients with low percentage of bone marrow CD34+ cells; moreover WT1 levels seem to be higher in patients with a worse R-IPSS. Most interestingly, leukemia evolution seems to be more frequent in patients with high WT1 expression (4 stable disease, 7 evolution) than in those with low Wt1 (17 stable disease, 5 evolution). Further statistical analysis is ongoing. We plan to extend this study to a larger number of patients to perform a stronger statistical analysis.

P019

NATURAL HISTORY OF HEREDITARY SPHEROCYTOSIS: A 20-YEAR STUDY

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Objectives: Hereditary spherocytosis (HS) is a congenital hemolytic anemia, due to defects of cytoskeletal proteins of the erytrocyte membrane, characterized by a variable degree of hemolysis, jaundice, splenomegaly, and cholelithiasis. Despite this recognized clinical presentation, there are few studies in adults that evaluate the natural history of the disease over a prolonged follow-up. *Materials and Methods:* In this retrospective single center study, 150 HS cases, F/M 77/74, (out of 480 diagnosed between 1974 and 2016) were followed for a median of 20 years (range 1-41) considering the presence of neonatal jaundice, splenomegaly, cholelithiasis, and occurrence of aplastic crisis, infections, and thrombosis. In addition, transfusion need, cholecistectomy, and splenectomy as well as blood counts, hemolytic indexes, and iron state were recorded at diagnosis and during the follow-up. *Results:* The median age at diagnosis was of 30 years with a wide range (1-73); splenomegaly was present in 80% of cases (median diameter of 17 cm), cholelithiasis in about a half, and neonatal jaundice in 1/3 of cases; 36 cases (24%) had been already splenectomized; and 33 (22%) cholecistectomized (11 concomitant to splenectomy). Of note, aplastic crisis had occurred in 7% of cases and transfusion need in 32% (Table). At diagnosis, the membrane defect was band-3 in 43%, spectrin in 31%, compound in 14% (spectrin/band 3+ankirin/band 4.2), or undetected in 7%. During the follow-up, 20 patients were splenectomized, of whom 9 before 2000, 8 between 2001-2010, 3 after 2010, and none in the last 3 years. Surgery was performed in 5 HS mild ,13 moderate and 2 severe; median Hb pre-splenectomy was 11.6 g/dL, and post splenectomy 15.3 g/dL; 31 underwent cholecistectomy (10 concomitant to splenectomy). Of note, thrombotic events were documented in 5%, all but one in splenectomized patients, and infections in 11% of cases. Ferritin values >500 ng/mL were observed in 23 cases (15.3%), of whom 18 had never been transfused; values >800 ng/mL were in 9 patients, all but one never transfused. *Conclusions:* our study confirms the great heterogeneity of the disease, the efficacy of splenectomy, which is however progressively abandoned. Hyperferritinemia is not infrequently and iron overload deserves consideration in these patients (independently from transfusion).

Table 1.

Natural History

	At diagnosis		At the last follow up	
	In splenectomized patients (n 36)	In non splenectomized patients (n 114)	In splenectomized patients (n 20)	In non splenectomized patients (n 94)
Laboratory data				
Hb (g/dL) median (range)	14.1 (5.2-18.4)	12.1 (6.4-16.4)	15.3 (9.1-18.2)	12.1 (8-16.2)
MCV (fl) median (range)	87 (68-112)	86 (65-102)	87.1 (76.6-93.3)	87.9 (63.5-100)
MCHC (g/dL) median (range)	34.9 (27-38.8)	35.7 (29.9-37.9)	35 (30.3-37.4)	35.6 (30-39.4)
Indirect Bilirubin (mg/dL) median (range)	0.9 (0.2-7.5)	1.8 (0.24-8.9)	0.6 (0.2-1.55)	2 (0.33-9.44)
Ret (x10^6/L) median (range)	88 (14-511)	231 (10-700)	109.5 (15.8-264)	235 (22-564)
Ferritin (ng/mL) median (range)	107 (4-1167)	174.5 (10-1403)	75.5 (10-792)	264 (21-4711)
Transferrin saturation (%) median (range)	36 (10-92)	29 (11-95)	27.9 (10.5-74.9)	29.5 (2.4-83.6)

Clinical data	At diagnosis	At the last follow-up
Neonatal Jaundice (n of pts, %)	48 (32%)	-
Aplastic crisis (n of pts, %)	11 (7.3%)	10 (6.6%)
Tranfusions (n of pts, %)	48 (32%)	14 (9.3%)
Exsanguinotrasfusion (n of pts, %)	7 (4.6%)	0
Splenomegaly (n of pts, %)	120 (80%)	86 (57.3%)
Splenic diameter (cm) median (range)	17.5 (11-25)	17(12-25)
Splenectomy (n of pts, %)	36 (24%)	20 (13.3%)
Cholelithiasis (n of pts, %)	71 (47.3%)	67 (44.6%)
Cholecystectomy (n of pts, %)	33 (22%)	31 (20.6%)
Infections (n of pts, %)	2 (1.3%)	17 (11.3%)
Thrombosis (n of pts, %)	3 (2%)	8 (5.3%)

P020

BREAKTHROUGH HEMOLYSIS CONTROLLED BY ECULIZUMAB ESCALATION DURING PREGNANCY IN PAROXYSMAL NOCTURAL HEMOGLOBINURIA (PNH): A SINGLE CASE REPORT

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Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired clonal stem cell disorder characterized by intravascular hemolysis, cytopenia and thrombophilia. Thromboembolism, infection and premature birth are main reasons for significantly increased maternal and fetal morbidity and mortality during pregnancy and the following post-partum period in PNH patients. Therefore PNH has been considered a relative contraindication for pregnancy. The terminal complement cascade inhibitor Eculizumab prevents fatal complications and nearly normalizes overall survival in PNH. Therefore it has become the standard treatment in patients with symptomatic PNH. However, there are limited published data regarding the use of Eculizumab during pregnancy, the postpartum period or, more less, during lactation. We report a 28 year old PNH patient who became pregnant while on Eculizumab. The therapy was continued throughout the whole pregnancy. At diagnosis of her pregnancy (6th week of gestation) anticoagulation therapy with low molecular weight heparin was initiated and continued although no clinical sign of thrombosis was present. She was immediately introduced in an interdisciplinary team for high risk pregnancies consisting of specialists for gynecology, internal medicine, anesthesia and hematology. During the third trimester of pregnancy she developed a breakthrough hemolysis in terms of symptomatic anemia requiring repeated blood transfusions. Hemolysis was successfully controlled by dose escalation of Eculizumab first from 900 mg to 1200 mg biweekly and consequently shortening the administration interval from a bi-weekly to a weekly scheme until the birth of the baby. She successfully delivered a healthy baby at term by natural birth without any complications. One month after delivery the patient has returned to her usual therapeutic Eculizumab regimen (900mg biweekly) and is breast feeding her baby. The anticoagulation treatment is still ongoing as recommended for the first three months. The baby girl is developing well according to her age. This case reports a favorable outcome of a PNH patient who became pregnant while under Eculizumab, supporting the scarce published experience that this drug can be given and can even be escalated during pregnancy in PNH patients.

P021

MANAGEMENT OF LOW-RISK MYELODYSPLASTIC SYNDROMES WITH ERYTHROPOIESIS STIMULATING AGENTS (ESAS) IN REAL-LIFE EXPERIENCE: AN UPDATE FROM RECAMDS (REGISTRO CAMPANO DELLE SINDROMI MIELODISPLASTICHE)

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Erythropoiesis stimulating agents (ESAs) are the frontline treatment in low-risk anemic MDS patients and an employment of this therapy in the earlier stage of the disease can delay the need for RBC transfusion, hypothetically by slowing the disease course. It's matter of debate whether the clinical response is a result of proliferation and maturation of the dysplastic clone or stimulation of residual normal erythropoiesis by ESAs. Macrocytosis is one of the cytological hallmarks of dyserithropoiesis: an analysis of the erythropoietic response to ESAs therapy in a cohort of anemic non trasfusion-dependent MDS patients, enrolled in a retrospective register, RECAMDS, was performed. 183 patients, treated with standard-dose ESAs, have been retrospectively analyzed (Table 1). Data analysis was performed, according to IWG 2006 criteria, at the baseline, after 3 and 6 months of continuous treatment, with a subanalysis of the patients according to WHO and R-IPSS risk stratification. ESAs were started at mean Hb concentration of 9.31 g/dl, mean serum EPO concentration: 51 mU/L, after a mean time from diagnosis of 6 months (r.1-118). Overall response rate(ORR) was 83.6% (153/183), no difference among WHO and IPSS subgroups was found: 132/183 (72.1%) achieved response after 3 months of treatment, while other 21/183 (11.2%) after 6 months. 19 patients with stable disease (non-responders, according to IWG criteria), in which treatment was continued, achieved response after 9 months. In the macrocytic-responders group 83.2% exhibits again macrocytosis after 3 months, while 16.8% become normocytic. In the normocytic-responders group 89.8% exhibits again normocytosis, while 10.2% become macrocytic: in these patients, after 3 months, there was a contemporary worsening in neutropenia and thrombocytopenia, with transfusion-dependence, regarded as first signs of progression of disease. Non-responders were 30/183 (16.3%): in the macrocytic non-responders group 89% exhibit again macrocytosis after 3 months, while 11% become normocytic; in the normocytic group 76% exhibits again macrocytosis, while 24% become normocytic. These preliminary data can suggest that, in the majority of MDS patients responsive to ESAs, the increase of Hb concentration occurs mainly stimulating erythroid production in MDS clones; in the minority of patients probably it happens recruiting residual polyclonal erythropoiesis. It is interesting to note that stimulating effects of ESAs last even when the expression of dysplasia progresses.

Table 1.

MDS PATIENTS	183
M	89 (49%)
F	94 (51%)
ERYTHROPOIESIS	
BASELINE HB (mean, g/dL)	9.31 g/dL (r. 7.1-11.3)
BASELINE SERUM EPO (mean, mU/mL)	51 mU/mL (r.3-84)
OVERALL RESPONSE RATIO	
RESPONDERS	153/183 (83.6%)
RESPONDERS AT 3 MONTHS	132/183 (72.1%)
RESPONDERS AT 6 MONTHS	21/183 (11.4%)
RESPONDERS AT 9 MONTHS (NON RESPONDERS IN IWG 2006)	19/183 (10.3%)
NON RESPONDERS	30/183 (16.3%)

Hemostasis and Thrombosis

P022

NONACOG BETA PEGOL FOR THE PROPHYLACTIC TREATMENT OF CHILDREN WITH HAEMOPHILIA B: INTERIM RESULTS FROM THE PARADIGM™5 CLINICAL TRIAL

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Introduction: Nonacog beta pegol (N9-GP) is a recombinant glycoPEGylated factor IX (FIX) developed for the treatment of haemophilia B with an extended half-life compared with conventional FIX products. Here we review new interim findings from the ongoing extension of paradigm™5, a non-controlled, phase 3 trial investigating the safety, efficacy and pharmacokinetics of N9-GP for the prophylaxis and treatment of bleeds in previously treated paediatric patients. *Methods:* The main trial enrolled and treated 25 children (aged ≤12 years) with haemophilia B (FIX ≤2%). Patients were stratified into two age groups: younger (0-6 years) and older (7-12 years) children; and received N9-GP 40 IU/kg once weekly for 52 weeks. A total of 22 patients entered the subsequent extension phase: 11 younger and 11 older children. Here we present the findings from a planned interim analysis, including all relevant exposures in the main and extension phases (cut-off date: 1 January 2016). *Results:* No patients developed inhibitors and no unexpected safety concerns were identified (mean treatment period: 2.55 years/patient; total in-trial exposure days: 3412 [136.5 per patient]). The mean N9-GP prophylaxis dose was 43.2 IU/kg and the mean annualised consumption was 2297.6 IU/kg/patient. Overall, 69 bleeds were reported in 19 patients, of which 37 bleeds occurred in 15 patients during the main phase (mean treatment period: 0.97 years/patient) and 32 bleeds occurred in 11 patients during the extension phase (1.80 years/patient). A majority of bleeds (60%) occurred >4 days after the last dose; two thirds of these (40% of all) were traumatic. The overall proportion of bleeds that showed a successful haemostatic response to treatment was 92.8% (94.4% and 92.2% in younger and older children, respectively) and 85.5% of bleeds resolved after one dose. The estimated mean annualised bleeding rate (ABR; bleeds per patient per year) was 1.08 (0.61 and 1.48 in younger and older children, respectively). The estimated mean ABR progressively decreased each 6-month period: 1.60, 1.50, 0.82, 0.82 and 0.33 at 1–6, 7–12, 13–18, 19–24 and >24 months, respectively. *Conclusions:* The latest data from the ongoing paradigm™5 extension trial confirm the longer-term safety and efficacy of N9-GP for the prevention and treatment of bleeds in children with haemophilia B.

P023

MANAGING CORONAROPATHY IN A PATIENT WITH ACUTE MYELOID LEUKEMIA UNDERGOING ALLOGENEIC BONE MARROW TRANSPLANT

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The association of heart failure and acute myeloid leukemia (AML) is a rare concomitant condition. Nevertheless, AML has a not negligible incidence in patients over 60yo, when coronary diseases reach their maximum prevalence. The management of coronaropathy in pts with AML represents a great challenge for physicians. Risk of bleeding is higher especially during prolonged aplasia. In January 2013 a 62 yo man was admitted to our hospital with a diagnosis of post-myelodisplastic AML. Bone marrow showed infiltration (24%) of undifferentiated

myeloid blast-like cells. Flow cytometry identified a percentage (10%) of immature elements CD34+/CD13+/CD33+/CD117+. No recurrent molecular markers for AML detected. Normal karyotype. The patient underwent a standard induction with cytarabine and idarubicin (7+3) and successively consolidation with cytarabine, idarubicin, etoposide (5 +3+3) chemotherapy. Because of the complete bone marrow response, the high prognostic risk and the availability of a full matched relative donor, he was candidate for allo-BMT. Cardiac ultrasound scan showed an ejection fraction lower than expected (34%). Coronary angiography revealed critic anterior descending coronary artery stenosis. He was treated with percutaneous transluminal angioplasty and bare-metal stenting (BMS). Given the high prognostic risk of the malignancy, we decided to confirm the allo-BMT program despite of the cardiac impairment. The choice of BMS allowed cardiologists to reduce duration of dual antiplatelet therapy. In August 2013 we conditioned the pt with the myeloablative regimen used at our center consisting of the triplet thiotepa, fludarabine, melphalan. Then he received a dose of 3,95 106/kg of CD34+ cells peripherally collected. Platelet (PLT) transfusions were given to maintain PLT count above 20 109/L to allow administration of anticoagulants (AC) (calcic nadroparin 0,4 ml once a day). No bleeding episodes were observed. At the last follow-up, 3 years after allo-BMT, the pt confirmed complete remission with a complete immunological recovery. He had no onset of further cardiovascular events. AML is a highly aggressive and often incurable malignancy. Survival is even worse in the elderly patients, in the post-myelodisplastic subgroup in which responses are often short-lived. The use of BMS, the reduced intensity conditioning and the maintenance of AC for PLT values above 20000/mcL may allow to extend allo-BMT in elderly pts with cardiovascular comorbidities.

P024

ACQUIRED FACTOR VIII INHIBITOR IN A CASE OF CHRONIC LYMPHOCITIC LEUKEMIA

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Acquired hemophilia A (AHA) and chronic lymphocytic leukemia (CLL) is an extremely rare concomitant condition. Nevertheless, clinical course of CLL may be complicated by autoimmune phenomena. In Sept 2015, a 39 yo woman was hospitalized because of fever. Patient (pt) gave a history of CLL previously treated with 4 cycles of fludarabine, cyclophosphamide (CY) and rituximab (RTX) regimen. Complete blood counts (CBC) showed decreased platelet counts; blood cultures were negative. Bone marrow analysis ruled out CLL progression. After a week of hospitalization she developed prolongation of aPTT (58,9 sec) not corrected via mixing with an equal volume of normal plasma for 2 hours. Further investigation demonstrated FVIII activity of 34.2% (reference range: 50-150) and the presence of FVIII inhibitor (1 Bethesda unit/ml). The pt underwent oral administration of Prednisone (PDN) 100mg daily. After hospital discharge she was monitored weekly with CBC and coagulation testing. Despite an initial reduction of aPTT she developed, a month after, diffused skin ecchymosis, metrorrhagia and prolonged aPTT (94 sec). She was for a second time hospitalized and bleeding was successfully handled with the administration of tranexamic acid and recombinant activated factor VII concentrate. FVIII activity was again 34.2% and FVIII inhibitor 20 Bethesda unit/ml. CY 50mg orally two times daily and RTX 375mg/sqm once weekly were initiated. After the 4th administration of RTX, aPTT and FVIII levels had normalized and FVIII inhibitor was undetectable. The pt underwent monthly maintainance with RTX 375mg/sqm for 5 months. At the last follow-up, 3 months ago, the pt maintained complete remission (CR). Clinical management of AHA can be very difficult especially in cases of underlying malignancy and concomitant sepsis. In case of incurable underlying disorders, antibody eradication represents a great challenge to physicians. Treatment is based on controlling of acute bleeding, treatment to raise FVIII, but overall on inhibitor removal. Responses obtained after front-line therapy (PDN with or without CY) are variable ranging between 30-60%. RTX improved this percentage (more than 90%) in pts resistant to other agents. The case report described is peculiar because our pt developed an autoimmune complication while CLL was in CR and there were no indication to chemotherapy. Prompt diagnosis and initiation of specific therapy represent a critical point to reduce morbidity and mortality.

P025

EFFICACY OF LOW DOSE OF THROMBOPOIETIN RECEPTOR AGONISTS (TPO-RA) AS MAINTENANCE THERAPY IN THE TREATMENT OF IMMUNE THROMBOCYTOPENIA (ITP)

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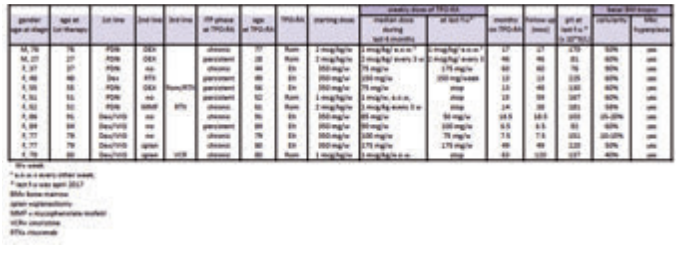
Background: The TPO-RAs romiplostim (R) and eltrombopag (E) are very effective in the treatment of steroid-refractory ITP. For both agents individualized dose regimens can be administered according to platelet (plt) count with R dose ranging from 1 to 10mcg/kg/week and E dose ranging from 25 to 75mg/day. However, since the main goal of ITP treatment is to achieve plt counts that prevents bleeding rather than 'normalizing' the plt count and individual response to therapy may vary, lower than expected dosages of either agent may be needed in a fraction of responding patients (pts). *Aim:* To describe a group of 12 non responding ITP pts who achieved maintenance of response on a modified TPO-RA regimen of R ≤1mcg/kg/week and E ≤175mg/week. *Patients and methods:* The present study is a retrospective case series of R or E-treated ITP pts who maintained a plt count >50x10/L at long-term follow up on reduced-dose regimen. The plt cut off for response was set at >50x10/L since it is high enough to allow pt's independence from strict laboratory and clinical monitoring. A "low-dose regimen" was arbitrarily defined as: a R dose ≤1mcg/kg/week and an E dose ≤175mg/week; no concomitant or rescue therapy; no bleeding events during the observation period. *Results:* A total of 12 pts out of a population of 64 TPO-RA treated patients (18.7%) meet the including criteria; demographics, clinical characteristics and therapy schedule are summarized in Table 1. No association between basal bone marrow cellularity, megakaryocyte hyperplasia, age and sustained response to low dose TPO-RA was found. 4/12 pts were able to discontinue the TPO-RA after a median of 14.5 mos on therapy. *Conclusions:* Since the main goal of ITP treatment is to achieve a stable plt count that prevents major bleeding rather than 'normalizing' the plt count, use of low-dose TPO-RAs seems a very promising strategy for the maintenance of response - almost in a quarter of pts in our series- and reduced therapy-related costs in ITP pts and it might also possibly improve safety. Of note, in a fraction of responding pts, low-dose TPO-RA may herald therapy discontinuation with sustained response maintained off any therapy.

Table 1.



P026

ABSOLUTE QUANTIFICATION OF JAK2V617F AND CALR MUTANT ALLELIC BURDEN IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS (MPNS) AND SPLANCHNIC VENOUS THROMBOSIS (SVT) BY USING DROPLET DIGITAL PCR (DDPCR)

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JAK2V617 and CALR mutations, can occur in Philadelphia-negative MPNs with a different incidence according to the type of disease and are risk factors for SVT independently of overt MPNs. The aim of this study was to evaluate the utility and efficiency of quantification of JAK2V617F and CALR mutant allelic burden in SVT patients tested by ddPCR compared to those obtained by conventional analytic methods. 132 pts (M/F: 68/64) with documented SVT, prospectively enrolled at our center between 1997 and 2015, were re-evaluated for JAK2V617F and CALR mutations by using ddPCR to quantify the mutate alleles in each patient. Based on the principle of sample partitioning into water-in-oil microdroplets, this method detects and quantifies somatic mutation with high selectivity and sensitivity. In details, ddPCR experiments were performed on DNA samples by using the Prime PCR ddPCR Mutation Detection Kit Assay for JAK2 wild-type and V617F mutation or CALR mutations (Bio-Rad), where wild-type and mutation probes were labeled with HEX or FAM fluorochromes, respectively, for JAK2 and CALR mutations. After amplification droplets were read in the QX 200 droplet-reader and analysis of ddPCR data was performed using the Quanta Soft analysis software. The percentage of JAK2V617F or CALR mutation was calculated as follows: positive droplets/positive droplets+wild-type positive droplets 100. JAK2V617F mutation was detected in 42 (31.8%) patients (PV: 8; ET: 8; PMF:14; unclassifiable MPNs: 12) by both restriction analysis and qPCR. In all patients mutation was in heterozygous state. Apart from showing 100% concordance with the previous JAK2V617F screening methods, ddPCR allowed us to quantify the mutate allele in all 42 positive patients at the diagnosis. In addition, by using ddPCR to analyze samples collected from the same patients during the clinical follow-up program, it was possible to evaluate JAK2V617F mutation changes over time in each positive patient. No mutations were found for CALR when we analyzed the samples for both ddPCR and conventional analytic methods. ddPCR is a suitable, precise, and sensitive method for absolute quantification of JAK2V617F mutant allelic burden in patients with SVT but not for CALR mutation, and may be useful for early disease detection and clinical management of patients without the need for a standard curve or comparison to a reference gene (limit of detention was 0.01% for both qPCR and ddPCR).

P027

PREGNANCY RELATED THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)

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TTP is a thrombotic microangiopathy characterized by severe ADAMTS13 deficiency, 90% secondary due to autoantibody and 10% congenital. In our series of 53 cases of TTP (40F/13M), 4 were pregnancy-related. One out of 36 non-pregnancy-related TTP was congenital (3%), whereas 3 out of 4 pregnancy-related TTP (75%) were congenital. Case 1: A woman with a previous non-pregnancy-related acquired TTP relapsed 10 years later, during her first pregnancy. A complete ADAMTS13 activity deficiency (<5%) was detected associated with inhibitors. Oral steroids and plasma exchange were started, with a significant clinical response. However, ADAMTS13 activity deficiency and antibody positivity persisted during remission. She delivered a premature baby at w. 25. Case 2: A woman with a previous history of HELP syndrome during pregnancy. She developed TTP at w. 29 of her second pregnancy. She had low ADAMTS13 activity levels and no inhibitors. Plasma treatment was started without achieving a complete remission. A cesarean section was performed at w. 32 with successful delivery of a normal child. A compound heterozygosity for two novel ADAMTS13 mutations was detected. Case 3: A woman with TTP during her first pregnancy (15th w.). She had a complete ADAMTS13 activity deficiency and no inhibitors. She received plasma infusion therapy plus steroids, and delivered on term a healthy baby. A compound homozygosity for two novel ADAMTS13 mutations was characterized. She had two subsequent pregnancies, and as a carrier of congenital TTP, was closely followed up for platelet count and hemolysis signs, and received prophylactic plasma infusions at lowering of platelet count. Pregnancy outcomes were successful on both cases. Case 4: A woman with TTP during puerperium (first pregnancy). After delivery, she developed severe thrombocytopenia, and had a complete ADAMTS13 activity deficiency with no inhibitors. Inherited severe ADAMTS13 deficiency was diagnosed by phenotype and genetic family analysis. Two years later, she was again pregnant. She was closely monitored for hemolysis signs and platelet count. She started plasma prophylaxis infusion when platelet count dropped <150x10^3/mmc, with a successful pregnancy outcome. Our data show that congenital TTP is more frequent in pregnant than non-pregnant women. A prompt diagnosis of congenital TTP is very important in order to start prophylactic measures (*i.e.* plasma infusion) to guarantee a positive pregnancy outcome.

P028

RUXOLITINIB AND VKA ALLOWS A FASTER RECANALIZATION IN PATIENT WIT SPLANCHNIC VEIN THROMBOSIS AND MYELOFIBROSIS

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Background: PMFoften causes SVT.Data regarding outcome of this subset of patients, mainly if treated with VKAplus ruxolitinib,are very few. *Aim:* Aim of this case series is to analyze if use of ruxolitinib+VKA is safe and effective in treatment of patients withPMF with IPSS INT-2. *Patients and methods:* This study is a retrospective study. 4 female patients, median age47(R35-55), withPMF INT-2 and with SVT(2portal, 2mesenteric+splenic+portal), median Hb11.5g/dl (R11-12.5), PLT90000/mcl (R70000-100000), WBC10000/mcl (R4000-11000), peripheral blood blasts 1%(R1-2), 1 patient heterozygous for factor V Leiden received ruxolitinib20mg/day+warfarin. All patients were Jak-2 mutated(GROUP1). In an historical cohort 6patients 2male,4female median age60(R45-65), 3PV,1ET,1PMF, 1 paroxysmal nocturnal hemoglobinuria and with SVT(4portal,2splenic+portal), median Hb12.5g/dl (R10-13.5), PLT150000/mcl(R110000-200000), WBC8000/mcl(R6000-10000), peripheral blood blasts 0%(R0-1), 1 patient heterozygous for factor V Leiden, 1 for prothrombin G20210, received Hydroxyurea 1000 mg(R500-1500)/day+warfarin. 3 patients were Jak-2 mutated (GROUP2). In another historical cohort 6patients 4male,2female median age60(R55-70), 3liver cirrhosis,3solid cancer and with SVT (5portal,1splenic+portal), median Hb11.5g/dl (R9-12.5), PLT100000/mcl (R90000-130000), WBC4000/mcl(R2000-9000), peripheral blood blasts 0%,received only warfarin. 1 patient was Jak-2 mutated(GROUP3). *Results:* Patients of GROUP1 showed a complete resolution of SVT in 2 cases and a partial portal recanalization in 2 cases after 3 months, without any thrombosis relapse or progression after 6 months; patients of GROUP2 showed a complete resolution of SVT in 2 cases, a partial portal recanalization in 2 cases and no resolution in 2 cases only after 6 months; patients of GROUP3 showed a partial portal recanalization in 3 cases, no resolution in 2 cases and 1 progression only after 6 months. MedianCRP after0, 3and6 months(mg/l) was respectively inGROUP1 27(R18-33), 8(R3-10), 6(R3-7), inGROUP2 30(R20-35), 18(R12-22), 10(R7-15), in GROUP3 38(R28-40), 35(R27-42), 33(R22-35). MedianCirculating Endothelial Cells/ml inGROUP1 after0, 3and6 months(mg/l) were respectively 1500(R800-5500),800(R600-1800),500(R400-1200). No patient showed side effects treatment related. *Conclusions:* Ruxolitinib and VKA is safe and effective and shows a faster recanalization and an antinflammatory effect in patients with PMF with SVT. These results needs confirmation on a larger cohort of patients.

P029

ARE THERE ANY DIFFERENCES IN PATIENTS WITH THROMBOPHILIC MUTATIONS OF PROTHROMBIN (FII) AND FACTOR V LEIDEN? THROMBOELASTOGRAPHY STUDY

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Factor II (G20210A) and factor V Leiden (G1691A) mutation have the same frequency in Deep Venous Thrombosis (DVT) and both are more common in youth and idiopathic DVT. In Pulmonary thromboembolism (PTE) these mutations have different incidence; particularly, Factor V is uncommon (factor V Leiden paradox). The cause of this phenomenon is not clear. It has been hypothesized that the clot has increased stability in patients (pts) with factor V mutation, but *in vitro* studies have not confirmed this hypothesis. Thromboelastography (TEG), thanks to a sequential evaluation of each phase of coagulation, could explain differences between carriers of the two mutations. We have extended the number of pts already studied with this *Methods:* TEG was performed in 11 pts with FII (G20210A) mutation, in 13 with FV (G1691A) mutation and in 5 controls, all of them with no history of thrombosis and therapy with anticoagulants or antiplatelet agents. TEG was performed with Haemoscope TEG analyzer, based on kaolin procedure. As in previous study, TEG parameters were in the normal range with the exception of some cases with R increased but we confirmed the differences of each parameter between the carriers of two mutations. Although we can not perform statistical analysis because of the sample size, we observed that pts with G20210A had increased R values (9,74 *vs* 9,28), higher K values (3,06 *vs* 2,51), lower maximum amplitude (65,7 *vs* 68,48), increased percentage of lysis at 30 and 60 min (1,26 *vs* 0,47 and 4,79 *vs* 3,01), CL lower (-3,37 *vs* -2,32). We can also observe that in some samples of G20210A carriers the percentage of lysis at 30 and / or 60 min is close to the upper limits of normal range, which does not occur in pts with V Leiden mutation. All these data could suggest a different relationship between coagulation and fibrinolysis in pts with the two mutations: pts with V Leiden mutation could have a more stable clot and less tendency to thrombus embolism. We need a prospective study in a larger cohort of pts and with most advanced methods to confirm these hypotheses. To date, we have studied only carriers of the two mutations with no history of thrombosis. It may be useful to evaluate also pts who are currently not on therapy but with pre-existing thrombosis. Study of pts at the onset of TVP could help to verify if there is a correlation between fibrinolytic activity at time of diagnosis and the risk of subsequent pulmonary embolism.

P030

SECOND LINE MULTIAGENT THERAPY IN IMMUNE THROMBOCYTOPENIA: A OBSERVATIONL STUDY

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Nelle piastrinopenie immuni (ITP)la somministrazione di più agenti è di frequente riscontro nella comune pratica clinica in relazione a insoddisfacente risposta a trattamento singolo e nel tentativo di determinare un più rapido incremento della conta piastrinica ,soprattutto nei casi di grave piastrinopenia (PLT<10000/mmc) e sintomi emorragici attivi.Scopo del presente studio è stato valutare l'outcome di trattamenti combinati di seconda linea in pazienti adulti affetti da ITP recidiva/refrattaria. Abbiamo valutato in maniera prospettica (follow-up a 12 mesi) i pazienti che dal Gennaio 2014 al Gennaio 2016 fossero stati ricoverati presso la nostra UO con diagnosi di piastrinopenia refrattaria/recidiva, conta piastrinica <10000/mmc e sanguinamenti attivi sottoposti, entro i primi quindici giorni dal ricovero, a trattamento specifico di seconda linea con combinazione dei seguenti agenti: steroide, immunoglobuline, anti-CD20 ,Romiplostin. Una coorte storica di pazienti con diagnosi di ITP recidiva/refrattaria, sottoposta a terapia con agente singolo è stata inoltre esaminata per valutare eventuali differenze nella prognosi a dodici mesi. Un totale di 18 pazienti (8F,10M, età media 50 aa, range:18-87aa), ha ricevuto trattamenti in combinazione: 10(55.5%) hanno ricevuto due agenti (Immunoglobuline ad alte dosi,1g x 2 e steroide ,prednisone 1mg/kg per quattro settimane), 6 (33.3%) tre agenti(Immunoglobuline, prednisone e Rituximab 375mg/mq a settimana x 4 settimane) e 2(11.1%)quattro agenti (Immunoglobuline, prednisone ,Rituximab e Romiplostin). Una percentuale non trascurabile di pazienti è stata sottoposta a terapia combinata con tre e quattro agenti. Il tasso di risposta è stato pari a 75%, con 60% di risposte complete. Il tempo mediano alla risposta è stato pari a quattro settimane. Il tasso di risposta è stato superiore per i pazienti con ITP recidive(8/18) anziché refrattarie(10/18). Nessun evento avverso è stato registrato durante terapia combinata. Alla comparazione con la coorte storica, è stata osservata una più rapida risposta completa nei pazienti trattati in combinazione rispetto ad agente singolo (mediana di risposta pari a 5.6 settimane, p=0.01). Al follow-up a dodici mesi non sono state riscontrate significative differenze tra le due coorti. La terapia combinata in seconda linea può rappresentare una valida opzione di trattamento per ITP volta ad un più rapido incremento della conta piastrinica.

P031

SWITCH OF TPO-MIMETICS IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA: FLORENCE MONOCENTRIC EXPERIENCE

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Primary immune thrombocytopenia (ITP) is an immune-mediated condition characterized by isolated thrombocytopenia, with peripheral blood platelet count of <100.000/l in the absence of an identifiable underlying cause of thrombocytopenia. Clinical studies in patients with ITP demonstrated that thrombopoietin (TPO) mimetics increase platelet production and can outpace platelet destruction. From November 2008 and February 2017, 65 patients were treated with TPO-mimetics with a median follow up of 29 months (1-96): 39 patients underwent therapy with Romiplostim and 26 to Eltrombopag. In our study we evaluated 18 patients who received both of therapies: among patients treated at first with Romiplostim 10 patients (9F; 1 M) switched to Eltrombopag and 8 patients (3 M; 5 F) switched from Eltrombopag to Romiplostim. In the group of 10 patients treated at first with Romiplostim, 5 patients started Eltrombopag because were no responders, 3 for loss of response and 2 patients because of adverse events. In the group of 8 patients at first treated with Eltrombopag, 4 patients didn't obtain any response with Eltrombopag and switched to Romiplostim, 1 for loss of response and 3 patients because of adverse events. Among patients switched from Romiplostim to Eltrombopag, 2 achieved complete response, 4 response and 4 were no responders; among patients switched from Eltrombopag to Romiplostim, 4 obteined complete response, 3 response, 1 was no responder. Romiplostim and Eltrombopag stimulate the TPO-R but have different mechanisms of action, therefore, in our limited experience switching from one thrombopoietic receptoragonist to the other could be beneficial in clinical practice for patients with severe chronic immune thrombocytopenia who failed to respond or experienced adverse events to the first.

P032

EFFICACY OF TPO-MIMETICS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Background: Romiplostim and Eltrombopag are second generation thrombopoietin receptor agonists (TPO-RAs), effective in 70%-80% of relapsed/resistant Immune Thrombocytopenic Purpura (ITP). *Aims:* we evaluated the efficacy of TPO-RA in patients with ITP. *Methods:* from November 2008 and April 2017 66 patients (34 M; 32 F) were treated with a median follow-up of 29 months (1-96): 39 underwent therapy with Romiplostim and 27 to Eltrombopag. In patients treated with Romiplostim, 21 had already received more than 4 lines of therapy, while in the case of Eltrombopag were 4/27. The median baseline platelet count was $21x10^9$/L (3-52) in Romiplostim group and $15x10^9$/L (1-53) in Eltrombopag group. *Results:* in patients treated with Romiplostim we observed 22 complete response and 10 response, with a response rate of 82%, while 7 patients were no responders. 26 (66%) stopped treatment after a median time of 16 months (1-93): 9 for stable response, 6 for no response, 3 for loss of response; 3 for adverse events, 2 underwent splenectomy, and 3 for other causes. In patients treated with Eltrombopag 17 achieved a complete response, 4 a response (total responses: 80%), 6 were no responders. 15 (55%) patients stopped Eltrombopag after a median time of 1,5 months (1-12): 6 for adverse events, 6 for no response, 1 for loss of response, 2 patients achieved a CR and interrupted treatment, maintaining a sustained remission. Platelets at follow-up are in patients who are still under treatment or stopped treatment are $161.5x10^9$/L (34-289) in Romiplostim group, and $139x10^9$/L (36-406) in Eltrombopag group. We observed that therapeutic response was influenced by the starting platelet count. In particular

platelets count before therapy influenced the first response observed. In particular in patients treated with Romiplostim PLT pre-treatment directly correlated with the first response and the maintenance of response during treatment at month 1°, 2° 3° and 6. Patients with a median starting platelet count of 15 10^9/L obtained a response (CR+R), while almost all patients who started therapy with PLT <15 10^9/L at baseline can obtain an initial response, but the majority is non-responder. *Conclusions:* TPO-mimetics have proved efficacy in patient with ITP and their use can be applied in several conditions (bridge to splenectomy; sustained response; switch and discontinuation). Future study on large series of patients are needed to best correlate baseline platelets with hematological response.

P033

HIGH-DOSE DEXAMETHASONE AND ELTROMBOPAG IN CHRONIC IMMUNE THROMBOCYTOPENIA (ITP): A SINGLE INSTITUTION EXPERIENCE

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Background: Eltrombopag is the first oral nonpeptide TPO-R agonist approved, as single agent for the treatment of chronic immune thrombocytopenia (ITP). However, information dealing with the concomitant use of eltrombopag and high-dose dexamethasone in patients with chronic ITP are limited. *Aims:* we assessed the efficacy and safety of eltrombopag in combination with high-dose dexamethasone in a consecutive, unselected cohort of chronic ITP patients treated at single institution in the period May 2014-March 2017. *Methods:* nineteen patients (11 F/ 8 M) with median age 53 years (range, 29-75) who had at least a 6-month history of ITP (median 26 months; range 6-220 months) were eligible for this retrospective evaluation. All patients had a platelet count lower than 30.000/μL and bleeding manifestations were present in 13 out of 19. No patient had an active infection, drug-associated thrombocytopenia, positive serology for HIV, hepatitis B or hepatitis C, malignant diseases or was pregnant. Eltrombopag treatment was initiated at 50mg once a day in all patients. Dose and schedule were individualized with the goal of achieving and maintaining platelets ≥50.000/μL, eltrombopag dose did not exceed 75mg/day. Dexamethasone 40mg/day was given for 4 days every 28 days. Patients with glucose intolerance or diabetes who needed therapy received mitigated dose of dexamethasone (20mg/day). Response and complete response (CR) required an increase of platelet count above 50.000/μL and 130.000/μL, respectively. *Results:* all patients achieved a response during the treatment while a CR was obtained in 16 of 19 patients. Maximum response was reached after a median time of 9 weeks (range, 2-39). After a median follow-up time of 66 weeks (range, 9-149) response was still maintained in all patients while five patients lost CR. Four patients who lost CR were receiving maintainence therapy with eltrombopag (50/75mg/day from a period ranging between 2 and 14 weeks). In the fifth patient CR was lost 7 weeks after treatment was interrupted because of pregnancy. *Conclusions:* results of this study although limited by the small sample size and the lack of a comparative randomized design suggest that high-dose dexamethasone in combination with a thrombopoiesis stimulating agent led to a long-lasting remission of ITP. Highdose dexamethasone may modify the immunological milieu, resulting in an enhanced response to the thrombopoietin receptor agonist.

P034

ROLE OF THE ADAMTS13/VWF COMPLEX IN THE THROMBOTIC AND BLEEDING EVENTS OF 67 PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA PROSPECTIVELY OBSERVED FOR 36 MONTHS

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Patients with Essential Thrombocythemia (ET) may have thrombotic and bleeding complications with increasing morbidity and mortality. Age, previous history of thrombosis, elevated white cell counts and JAK2 allele burden are considered risk factors for both arterial (ATE) and venous (VTE) thrombosis while elevated platelet counts and quantitative/qualitative defects of the von Willebrand factor (VWF) are associated with bleeds. To evaluate the role of the complex ADAMTS13/VWF in thrombosis and bleeds, 67 patients with ET diagnosed according to WHO criteria have been observed prospectively for thrombotic and bleeding events during a 36-month follow-up. Patients enrolled (n=67) were exposed to the diagnostic tests needed to characterize ET but signed an informed consent for these ADAMTS13/VWF evaluations. VWF:RCo, VWF:Ag) activities were measured by the automatic ACL-TOP systems while ADAMTS-13 was tested by TECHNOZYM activity ELISA. These data were expressed *versus* International Standard in IU/dL. The VWF:RCo/Ag ratios were calculated as surrogate marker for the loss of the HMW-VWF multimers but such VWF defect were also confirmed using agarose-gel electrophoresis. Statistical analyses among the cases with or without complications were performed by SPSS. Thrombotic [ATE(7), VTE(10)] and bleeding [Menorrhagia(4), Epistaxis(4), Gastrointestinal(2)] complications were observed in 17/67(25%) ET-T and 10/67(15%) ET-B cases respectively while 40/67(60%) ET patients did show no thrombotic/bleeding events (ET-NTB). The clinical-lab data [median(range) of ET-T or ET-B were compared with those found in ET-NTB. Age, sex, JAK2 allele burden and white cell counts were not different (p=0.18) among the 3 groups. In ET-T(n=17), VWF:ACT(U/dL) was higher [162(96-209)] than that of ET-NTB[109(76-239)] with ADAMTS13(U/dL) lower [65(41-142)] than that of ET-NTB [87(56-164)]with p<0.05. In ET-B(n=10), Plt-counts(/uL) were higher [722(478-1342) than those with ET-NTB[464(390-830) with VWF:ACT(U/dL) lower [53(27-92)] than that of ET-NTB (p<0.05). In ET-B, there was also a loss of the HMW-VWF multimers assessed by SDS-agarose gel electrophoresis in most cases as predicted by VWF:ACT/Ag ratios <0.6. ADAMTS13 levels [85(50-154) were not different from ET-NTB. Based on these results from a relatively small number but well characterized patients, we can suggest that the ADAMTS13/VWF complex might be considered an additional risk factor to predict thrombotic or bleeding events in ET.

Infections

P035

THE PREDICTIVE VALUE OF ASPERGILLUS PCR TESTING ON BRONCHOALVEOLAR LAVAGE FLUID FOR EARLY DIAGNOSIS OF INVASIVE PULMONARY ASPERGILLOSIS IN HEMATOLOGIC PATIENTS

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Despite serum and bronchoalveolar lavage fluid (BAL) galactomannan (GM) are recommended as indirect mycological tests for diagnosis of probable invasive pulmonary aspergillosis (IPA) by EORTC/MSG guidelines, the putative value of Aspergillus PCR on BAL for the diagnosis of IPA still remains to be determined. Based on these considerations, we performed a prospective study on hematologic patients, aimed at: evaluate the role of Aspergillus PCR detection on BAL for the diagnosis of IPA in comparison with standard mycological indirect tests; evaluate the efficacy of bronchoscopy with BAL as systematic diagnostic approach of lung infiltrates (LI). Bronchoscopy was performed in all hospitalized patient with diagnosis of acute leukemia and LI at onset of disease before therapy start, and in any other hematologic patient in any phase of disease with LI requiring hospitalization because of febrile neutropenia and/or respiratory distress not responding to broad-spectrum antibiotics. In all cases we performed the same diagnostic work-up including blood-swabs cultures, serum galactomannan (GM) and beta-D-glucan, serum PCR for CMV; BAL fluid was studied by cultures, GM and PCR for S pneumoniae, L pneumophila, C pneumoniae, M pneumoniae, B pertussis and parapertussis, H influenziae, respiratory virus and CMV, P jiroveci, M tuberculosis, Nocardia spp, L monocitogenes and Aspergillus spp. Out of 769 patients admitted in our ward from April 2013 to October 2016, 85 had LI and 47 of them underwent BAL (total procedures: 51). A causal agent of LI was detected in 33 cases (65%) allowing to modify the ongoing anti-microbial treatment in 25 of these ones (76%). Twelve cases of probable IPA, according to EORTC/MSG criteria, were diagnosed. In addition, we found 7 cases of LI with radiologic criteria suggestive for IPA but negative by serological tests and GM on BAL, in which, however, the positive results gathered by Aspergillus PCR determination were followed by the administration of voriconazole, after considering such a result as an "indirect" mycological criterion. All these 7 cases obtained a satisfactory clinical outcome with complete LI resolution, as documented by CT assessment. One life-threatening post-procedure complication was observed. In conclusion, maybe the time has come to include Aspergillus PCR among the indirect mycological tests of probable IPA, and to consider BAL as an indispensable and safe diagnostic approach to LI also in difficult hematologic patients.

P036

GLOBAL APPROACH TO MULTI-DRUG RESISTANT PSEUDOMONAS AERUGINOSA (MPA) OUTBREAK IN A HAEMATOLOGY WARD (HW): EPIDEMIOLOGY, MANAGEMENT AND INFECTION CONTROL MEASURES

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Background: MPA is associated with high mortality and morbidity rates in immunocompromised patients (pts). From 01/2007 to 12/2008 MPA incidence in our HW has been 9% (4,5% year). During the period 01/2009-04/2013 an outbreak developed. *Aims:* To describe the MPA outbreak. *Methods:* retrospective study: from 01/2009 to 04/2013 we hospitalized 415 adult pts; of these, 106, at high infectious risk (HIR) for severe and prolonged expected post-chemotherapy neutropenia, have been routinely screened at admission with microbiological samples (nasal, pharyngeal and rectal swabs) and additional tests when clinically indicated. During this period, we observed a dramatic incidence of MPA isolates. As a consequence, we fulfilled specific sequential measures, to assess potential reservoirs and breaks in infection control and to manage the outbreak, summarized by the following 4 phases: phase A: closing of HW from 29/04/2013 to 09/06/2013; phase B: serial pre and post-disinfection environmental swab sampling from each room concerning: sink filters, bidet filter, shower filter, flush button, infusion pump, TV remote control, 70% ethylic-alcohol gel bottle, floor sink, bedpan as well as water samples from bedpan automatic washers (BAW); phase C: room environmental disinfection and microbial decontamination with nebulized H(2)O(2) solution added with silver cations; phase D: prohibition of the use of BAW, introducing the use of disposable bedpans and planning an environmental sampling and disinfection program. *Results:* Retrospective study data: 82 pts carried bacterial isolates: 48 (59%) had MPA, classified as colonisation in 13 pts (mainly detected on rectal swabs) and true infection in 35 (10 pneumonias (29%), 6 anorectal/perineal (17%), 5 urinary tract (14%), 14 (40%) bloodstream infections). Ten pts died of MPA related infection, with a mortality of 53% (10 on 19 pts). Phase B defined a prevalence of PA isolates in 6 out of 7 rooms in different sample types, with 4 MPA isolates identified in 3 different BAW and 1 bedpan after washing cycle. After phase C, a new phase B demonstrated sterilization of 3 out of 6 PA and 3 out of 4 MPA isolation sites. As a main corrective action, after 41 days, we resumed admissions and approached phase D, resulting into a prompt and maintained decrease in isolates (Table 1). *Conclusions:* Our experience highlights the value of environmental and personal hygiene measures on MPA infections control.

Table 1. MPA isolates and mortality rate after phase D.

	2013 (second half)	2014	2015	2016
HIR pts	**35**	**38**	**51**	**52**
Colonisation (%)	**0**	**1**(3)	**0**	**0**
Infection (%)	**2**(6)	**1**(3)	**1**(2)	**0**
Mortality (%)	**0**	**0**	**0**	**0**

P037

SEIFEM 2016 STUDY: INCIDENCE OF PROBABLE AND PROVEN INVASIVE ASPERGILLOSIS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA DURING CONSOLIDATION THERAPY

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Aims: To evaluate incidence and mortality for invasive aspergillosis (IA) in acute myeloid leukemia AML patients during the consolidation phase. *Methods:* All cases of proven/probable IA observed during consolidation chemotherapy in adult and pediatric patients with AML between 2011 and 2015 were retrospectively collected in a multicenter study involving 37 Italian hematologic centers. All relevant clinical data were collected in CRFs.

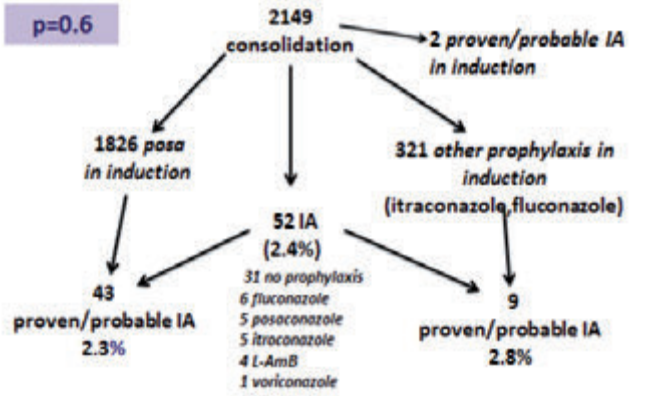

Figure 1.

Results: Overall, information about 2149 cases of AML was collected. As a consolidation, all the patients received high/intermediate doses cytarabine, in combination with an anthracycline in 30. A total of 52 patients (2.4%) developed an IA [13 proven (0,6%) and 39 probable (1.8%)]. Male/female ratio was 33/19, median age was 57 yrs (range 5-79). Twenty-one of 52 (40%) were on antifungal prophylaxis at the time of IA diagnosis: posaconazole in 5 (24%), fluconazole in 6 (28%), itraconazole in 5 (24%), liposomal amphotericin B (L-AmB) in 4 (19%) and voriconazole in 1 (5%). In 2 of 52 IA was a reactivation of an infection diagnosed during induction (Figure 1). Empiric antifungal therapy (AT) was initiated in 33 (65%) cases (mainly L-AmB: 24/33, 73%), while the remaining 19 (35%) received a pre-emptive/targeted approach. Fourteen cases switched from L-AmB to voriconazole, so that the most frequently targeted AT was voriconazole (29/52, 55%). The median duration of AT was 89 days (range 12-700). The overall mortality at day 120 was 19% (10/52) while the attributable mortality was 3.2% (5/52). During induction, many patients (85%) received posaconazole as antifungal prophylaxis while the remaining 15% received other antifungals. (Figure) In consideration of the hypothesized long-lasting activity of posaconazole, we also performed an analysis of incidence of IA during consolidation taking into whether or not the patients received posaconazole during induction. No statistical differences were observed between those receiving in induction posaconazole or other prophylaxis. (Figure). *Conclusions:* our study shows that the incidence of IA during the consolidation in patients with AML is low. Similarly, also the attributable mortality is low, likely due to advances in diagnosis and AT. The long-lasting benefits of posaconazole is not confirmed but it might be correlated to the few cases of IA observed.

P038

SEIFEM 2016 STUDY: INCIDENCE AND MORTALITY OF PROBABLE AND PROVEN INVASIVE ASPERGILLOSIS DURING SALVAGE TREATMENT OF PATIENTS WITH RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA

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Aims: To evaluate incidence and mortality of invasive aspergillosis (IA) during salvage treatment of patients with relapsed/refractory acute myeloid leukemia patients (AMLs). *Methods:* All cases of proven/probable IA observed during salvage chemotherapy in adult and pediatric patients with AMLs between 2011 and 2015 were retrospectively collected in a multicenter study involving 38 Italian hematologic centers. All relevant clinical data were collected in CRFs. *Results:* Overall 1350

cases of relapsed/refractory AMLs were collected. A total of 69 patients (2,4%) developed an IA [15 proven (1,1%) and 54 probable (4%)]. Male/female was 39/30, median was age 56 years (range 8-80). Thirty-three (48%) of 54 patients received fludarabine/cytarabine based regimens. A history of IA during first-line therapy was observed in 11/69 (16%) patients. All of these were on antifungals at the time of the IA reactivation: posaconazole in 3 (27%), fluconazole in 2 (18%), itraconazole in 1 (9%), and others in 5 (45%). Of the remaining 58 patients, 44 (76%) were on some form of treatment: posaconazole in 20 (45%), fluconazole in 9 (20%), itraconazole in 8 (18%), liposomal amphotericin B (L-AmB) in 3 (6%) and others in 4 (9%) (p=.04). Proven IA were associated with comorbidities such as diabetes (p=.02). Lungs were the most frequently affected site (63/69, 94%). Antifungal therapy (AT) was given in 67 of 69 cases. Empiric AT was initiated in 45 (67%) cases (mainly L-AmB: 32/45, 71%), while the remaining 22 (33%) received a pre-emptive/targeted approach. Eighteen patients switched from L-AmB to voriconazole, so that the most frequently targeted AT was voriconazole (45/67, 67%). The median duration of antifungal therapy was 35 days (range 2-1065). The overall mortality at day 120 was 39% (27/69) while the attributable mortality was 25% (17/69). No significant differences in mortality at day 120 and attributable mortality were observed between patients with or without previous IA [45%(5/11) *vs* 37%(22/58) and 27%(3/11) *vs* 24%(14/58), p=ns]. *Conclusions:* our study confirmed that in patients with relapsed/refractory AMLs the incidence of AI is low but the attributable mortality remains dramatically high regardless of a previous history of IA. These data suggest that also a very effective antifungal treatment is not able to reverse the unfavorable outcome of IA occurring in patients with a profoundly compromised immune system such as the one of patients with late stage AML.

P039

FUNGAL INFECTIONS OF THE CENTRAL NERVOUS SYSTEM AND PARANASAL SINUSES IN HEMATOLOGIC PATIENTS. A SEIFEM GROUP STUDY REPORTING DIAGNOSTIC-THERAPEUTIC APPROACH AND OUTCOME IN 87 CASES

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Introduction: Fungal Infections (FI) of the Central Nervous System (FI-CNS) and Paranasal Sinuses (FI-PS) are rare life-threatening infections in hematologic patients (pts) and their management remains a challenge despite availability of new diagnostic techniques and novel antifungal agents. In addition, analyses of large cohort of pts focusing on these rare FI, are still lacking. *Patients and Results:* Between January 2010 and December 2016, 87 cases of Proven (51) or Probable (36) FI-CNS (69/87) or FI-PS (18/87) were collected in 26 Hematological Centers. Median age of pts was 40 yrs (3-79) and 22/87-25% had less than 18 yrs. Acute Leukemia (AML or ALL) was the most common underlying disease (59/87, 68%) and 25/87-29% cases received a previous Allo-SCT. Aspergillus sp was the most common pathogen (61 cases, 70%), followed by Zygomycetes (18 cases, 21%), Criptococcus sp (4 cases, 5%) and Fusarium sp (2 cases, 2%). The lung was the primary focus of FI in 48% of cases. The CNS biopsy was performed in 7/69-10% of FI-CNS cases and a sinus biopsy was done in 10/18-56% of FI-PS (p=0,03). The GM Test on cerebrospinal fluid (CSF) has been performed in 41% of FI-CNS (28/69) and it was positive in 20/28-71%. Eighty-four pts received a first line antifungal therapy (AT) with Amphotericine B (mainly L-AMB) in 56/84-67% of cases and Voriconazole in 35/84-42%. Moreover, 56% of pts received 2 or more lines of AT and 43% were treated with a combination of 2 or more antifungal drugs. The median duration of AT was 58 days (range 1-835). A surgical intervention was performed in 21/87-24% of cases but only 7/69-10% of FI-CNS underwent neurosurgical intervention. The ORR to treatment (complete and partial responses) was 56% (CR 34% and PR 22%). The median OS after FI-CNS and FI-PS was 4,7 and 3,3 mths, respectively (P=ns). One year OS was 31% without significant differences between FI-CNS and FI-PS. Outcome according a multifactor risk analysis (including age, status and type of underling disease, allo-SCT, PMN at baseline and recovery, lines and duration of AT, surgical intervention, etc) has been evaluated. The FI attributable mortality was 33%. *Conclusions:* Mortality of FI-CNS/PS remains high but, compared to previous historical data, it seems to be reduced probably due to the availability of new antifungal drugs. The results arising from the analysis of this large cohort of cases, may allow a more effective management of these rare FI complications in hematologic pts.

P040

BENDAMUSTINE TREATMENT FOR LYMPHOPROLIFERATIVE DISORDERS IS ASSOCIATED WITH HIGH INCIDENCE OF CMV REACTIVATION

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Bendamustine (BENDA) alone or in combination with other drugs is being increasingly used in the treatment of chronic lymphocytic leukemia (CLL), non-Hodgkin lymphomas (NHL), and multiple myeloma (MM) due to its safety and efficacy. Variable infection rates both in BENDA monotherapy and in BENDA-containing regimens have been documented. Limited data are available on cytomegalovirus (CMV) reactivations and infections in patients with hematologic malignancies undergoing BENDA therapy. We retrospectively analysed for CMV reactivation 104 consecutive patients who underwent therapy with BENDA alone (4%) or combined with rituximab (96%). Our cohort included 80 B-NHL (38 indolent e 42 aggressive B-NHL), 4 T-NHL, 19 CLL, 1 MM with a median age of 71 years (range, 52–88) and a female to male ratio of 0.68 (male/female: 62/42). BENDA was used as first line therapy in 75% of patients and as salvage therapy in 25%. CMV monitoring was performed on plasma samples by a real-time TaqMan CMV-DNA polymerase chain reaction (PCR) assay, according to the manufacturers' recommendations (Roche). Quantitative CMV-DNA was detected every 3 weeks before starting the next BENDA cycle, while CMV-DNA monitoring was performed once a week in cases of CMV reactivation defined by plasma CMV-DNA levels higher than 137UI/ml. CMV reactivation was detected in 20 patients (19%) among the 105 patients analysed for CMV-DNA PCR, with a median CMV peak viral load of 3610 UI/ml, CMV reactivation occurred after a median of 50 days (range: 11-300) post-BENDA in 18 B-NHL (5 aggressive and 13 indolent NHL; 13% and 34%, respectively), 1 T-NHL (25%) and 1 CLL (5%), respectively. Oral valganciclovir (VGCV), a prodrug of intravenous GCV, at a dose of 450 mg twice daily determined a prompt clearance of CMV viremia in a median time of 30±13 days (range: 17-70) in 12 patients with CMV reactivation. Eight patients with CMV symptomatic infection post-BENDA were successful treated with oral VGCV at a dose of 900 mg twice daily for at least tot days, except for two cases developing fatal CMV disease. All patients showed a deep

lymphocytopenia at the time of CMV reactivation with a median of 769 lymphocytes/mmc. This single-center study provides further evidence that BENDA regimens for lymphoproliferative diseases are associated with a high CMV reactivation rate and therefore require routine monitoring of CMV surveillance to quickly start CMV-preemptive therapy.

P041

MALNOURISHED AND NEW THERAPEUTIC TARGET

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Introduction: According to recent data of Food and Agriculture Organization (FAO, 2015) there are more than 795 million people suffering from malnutrition around the world. It can lead the patient to death, mainly due to the severity of the infection. Deficiency in the leptin concentration is indicated as one of the main triggers of infection. In addition, serum leptin is considered as a biomarker of malnutrition, especially in elderly patients with leptinemia. Previous studies in our group have demonstrated that a synthetic leptin fragment, DELTA*, have shown bioactivity by inducing proliferation of hematopoietic stem cells. *Materials and Methods:* C57BL/6 mice were submitted to a normal protein feed intake containing 12% of protein (control CTRL), and a hypoproteic feed intake containing 2% of protein (malnourished – MALN). After 5 weeks of inducting protein malnutrition, one part of the malnourished animals received DELTA for 3 days (MALN+DELTA). The hemogram, immunophenotyping by flow cytometry of bone marrow, spleen and thymus cells, as well as cell viability, were evaluated after DELTA treatment induction. *Results and Discussion:* The DELTA fragment demonstrated its bioactivity by modulates the anemia in MALN+DELTA animal models. This treatment induces increase in total leukocytes production by proliferation and differentiation of hematopoietic stem cells. The treatment induces terminal differentiation of granulocytes and T lymphocytes in bone marrow, and it also increase T lymphocytes subtypes by inducing migration to thymus. Thymus immunophenotyping revealed increased T lymphocyte subtypes in the animal group MALN+DELTA compared to the MALN group. This effect was not observed in splenic cells, probably due to the short treatment period with DELTA. Finally, the cell viability analysis of bone marrow after DELTA treatment does not induces cell death. Therapeutic Potential: Synthetic fragments are inexpensive and quick to produce on a large scale. In addition, patients with HIV, adults and children living in regions of extreme poverty, all presenting infection, could be candidates for treatment with a synthetic leptin fragment DELTA.*The synthetic leptin fragment DELTA is in the patent process.

P042

DIAGNOSIS OF INVASIVE ASPERGILLOSIS IN HEMATOLOGICAL MALIGNANCIES: THE VALUE OF GALACTOMANNAN TEST PERFORMED IN BOTH BRONCHOASPIRATE AND PERIPHERAL BLOOD

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Background: Invasive aspergillosis (IA) represents a major cause of mortality in hematological malignant patients. Positivity of galactomannan antigen (GM) associated with typical pulmonary infiltrates allows an early diagnosis of IA and the initiation of specifical antifungal therapy. GM can be tested on peripheral blood serum (PB) and on bronchoalveolar lavage/aspirate (BAL/BA) with non-univocal results in different series. BA is less invasive than BAL because of a minor instillation of water in bronchus, easier to perform in hematological patients frequently presenting thrombocytopenia and respiratory failure. *Aims:* To compare the sensitivity of GM test both on BA and on PB in patients affected by hematological malignancies with suspected IA. *Methods:* Patients with suspected IA observed at chest CT scan underwent to BA from March 2012 to April 2017. Samples collected by BA and PB were analyzed for both culture exam and GM antigen. *Results:* We performed 48 BA in 38 patients (median age 60, range 25-78). GM was tested both in BA and in PB in 35 procedures: 25 AML, 4 NHL, 1 MDS, 1 AHA and 4 MM. In all patients also culture tests for bacterial or fungal infections were performed. GM resulted positive in 20/35 cases which performed BA. Among these 20, positivity in PB was found only in 2. The 20 GM positive cases on BA presented culture tests positive for Aspergillous in 2 patients, while 1 patient resulted affected by lung Mucormycosis infection. In 15/35 BA GM resulted not detectable, all serum GM performed in these 15 cases resulted negative, while one patient presented Aspergillous at culture test. The antifungal prophylaxis was ongoing on 11/20 patients resulted by BA GM positive and in 7/15 patients GM negative. To be noticed that the BA were performed bedside in 17/35 patients without any complication. *Conclusions:* In our experience BA proved to be an important and sensitive instrument for diagnosis of IA and is particularly useful in hematological malignant patients with thrombocytopenia and respiratory failure. Complications were not observed and the procedure can be performed at bedside. In our series of patients, the detection of GM by PB frequently failed to be diagnostic especially in those on antifungal prophylaxis, suggesting that only BA may be performed for early diagnosis of IA.

P043

CARBAPENEMASE-PRODUCING KLEBSIELLA PNEUMONIAE (KPC) COLONIZATION IN PATIENTS UNDERGOING ALLOGENEIC STEM-CELL TRANSPLANTATION: BEHAVIORAL MANAGEMENT AND ANTIMICROBIAL STRATEGY IN A SINGLE-CENTER EXPERIENCE

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Carbapenemase-producing Klebsiella pneumoniae (KPC) infections are an emerging cause of death after hematopoietic stem cell transplantation (HSCT). Around 2% of patients undergoing allogeneic-HSCT develop KPC infection; the colonization documented on rectal or on other swabs is followed by infection in up to 40% of the patients, with a mortality rate higher than 60%. We retrospectively evaluated all patients receiving a transplant from an allogeneic source between January 2011 (date of the first KPC detection at our Center) and January 2017. Eighty-five patients underwent HSCT, and 11 of them (12,9%) had rectal screening positive for KPC (5 acute myeloid leukemias, 2 acute lymphoid leukemias, 1 multiple myeloma, 1 myelodisplastic syndrome, 1 Chronic Myelo-monocytic leukemia, 1 mantle cell lymphoma). Six of these patients received myeloablative conditioning (MAC) and 5 received reduced-intensity conditioning (RIC). Contact precautions and hygienic measures were adopted in all carriers to limit the spread of the contamination. KPC-positive patients with febrile neutropenia were immediately treated with intravenous gentamycin, one of the few antimicrobial agents that maintains sensitivity against KPC. Seven out of 11 KPC-positive patients (63,6%) died: six of them (all patients who developed blood stream infection) died for KPC sepsis (3 after transplant, 3 after salvage therapy due to relapse). Four patients did not develop KPC sepsis, and they were alive at the last follow-up. The impact of KPC sepsis on transplant-related causes was 10%, with an incidence of 23% on all deaths due to infection. No statistical differences were observable between KPC positive and negative patients in transplant-related mortality at 12-months. Conversely, the KPC positivity conferred a high risk of infectious death after salvage therapy: three out of 4 relapsed KPC-positive patients died for KPC sepsis, whereas 13 out of 15 relapsed KPC-negative patients died for progression of disease. We can conclude that infection and colonization by KPC may represent a challenging problem in transplant recipients for the management of infectious complications and also for the eligibility to transplant in patients who acquire the pathogen before the procedure. Early diagnosis, contact precautions and empiric antimicrobial therapy are important measures to improve patients survival and to limit the outbreak. However, new decontamination strategies and antibiotics are needed.

P044

A RETROSPECTIVE STUDY ON INCIDENCE OF BLOODSTREAM INFECTIONS IN TWO DIFFERENT TYPES OF CENTRAL VENOUS ACCESS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background: The onco-hematological patients, because of the diseases they are suffering from, require a safe central vein vascular access (CVC), but its use can cause many local and systemic infectious complications. There are many CVC related infections including phlebitis, tunnel infections, the CVC pouch infection, bacteremia and sepsis. These events, prolong the length and costs of hospitalization as well as the the risk of mortality. For more than 30 years, in our Department, the most commonly used CVC type has been the tunneled Groshong®. Since 2009 it has been considered the opportunity and the chance to use a Peripherally Inserted Central Catheter (PICC), theoretically associated, with less infectious complications. *Patients and Methods:* We analysed the results of blood cultures from CVC that were positive for any microorganism both in and out-patients setting between January of 2011 to December of 2012. During this period, 145 tunneled Groshong® type CVC and 231 PICC were positioned, respectively. *Results:* The total number of positive cultures was 113 of which 69 isolated from tunneled CVC while 44 from PICC (61% *vs* 49%). The major incidence of blood cultures positivity we observed in patients with tunneled Groshong® type CVC was constant during the study period since the cases of positive blood cultures from tunneled Groshong® type and PICC were 44 and 20 cases (51% *vs* 19%) in the first year, and 25 and 24 cases (42% *vs* 18%) during the second year, respectively. Regarding the main microorganisms involved, the characterization of the blood cultures shows: Staphylococcus coagulasi neg. (35 cases), Escherichia coli (29 cases), Streptococcus alpha haemoliticus (12 cases), Pseudomonas aeruginosa (9 cases) and Enterococcus faecium/faecalis (5 cases). In all cases the incidence of positive cultures was major in tunnelled CVC respect to PICC. *Conclusions:* Although bloodstream infections remain a major complication of any type of CVC, our study supports the notion that bacteremias are less frequent when using a PICC compared to tunneled Groshong® type CVC. Future studies will be necessary to investigate whether other strategies based for example on the use of catheters with antimicrobial impregnation, new type of lock solutions or patients' dressing, could be effective to further reduce the incidence and severity of CVC related infections.

Acute Leukemias 1

P045

FEW-LAYER GRAPHENE SELECTIVELY KILLS MONOCYTIC NEOPLASTIC CELLS FROM PATIENTS WITH AML AND CMML

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Acute myeloid leukaemia (AML) in the M4 and M5 variants and chronic myelomonocytic leukaemia (CMML) are two subtypes of myeloid neoplasms both characterized by circulating neoplastic cells of the monocytic lineage. Traditional therapies are often limited by inadequate drug concentrations reaching the tumor site, intolerable cytotoxicity and resistance. In this scenario, nanotechnology could offer a new perspective to treat leukaemias more selectively, reducing undesired side effects. In this work, we studied few-layer graphene (FLG) dispersions and discovered a highly specific toxicity on human monocytes. Based on this interesting result, we evaluated the killing activity of graphene on monocytic neoplastic cells from a cohort of AML and CMML patients. We first analyzed by flow cytometry the impact of FLG on peripheral T, B, NK, dendritic cells and monocytes from healthy controls, showing a specific cytotoxic activity on monocytes, while all the other cell subtypes remained unchanged. The percentage of necrotic monocytes increased from 6% to 29%, 36% and 71% after 1, 4 and 24 h, respectively. The high selective capacity of our FLG to kill human monocytes could be potentially promising for treating leukaemias presenting high percentages of circulating monocytic neoplastic cells. Therefore PBMCs from a cohort of 7 patients with AML (M4 or M5) or CMML were treated with increasing doses of FLG to assess the capacity of graphene to specifically kill the neoplastic monocytes. The presence of FLG accumulated into the neoplastic cells was first observed on the peripheral blood smear of our patients. After treatment with FLG, the cancer cells were strongly affected in all patients in a FLG concentration dependent manner with no effect on other immune cells. The number of neoplastic cells was dramatically reduced even at very low concentrations, while at 25 and 50 microgram mL^{-1}, the percentage of cells was almost ablated from an average of 24% to 2.2% and 1.6%, respectively. Furthermore, we compared the specific effect of FLG with etoposide, a common chemotherapeutic agent, which conversely induced a significant T, B and NK cell toxicity. These findings open the way to a possible application of FLG as a specifically targeted tool against neoplastic cells in AML and CMML. This new therapeutic strategy based on graphene might be extremely advantageous over traditional chemotherapeutic treatments that are non specific and often impair all immune cell subpopulations.

P046

HYPOMETHYLATING AGENTS ± DLI AS SALVAGE THERAPY FOR ACUTE MYELOID LEUKEMIA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION. A REAL LIFE EXPERIENCE

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Introduction: Acute Myeloid Leukemia (AML) relapsing after allogeneic stem cell transplantation (Allo-SCT) remains a therapeutic challenge, with a very poor prognosis and limited therapeutic options outside clinical trials. *Patients and Results:* We report the outcome of 17 patients with AML relapsed after Allo-SCT and treated with hypomethylating agents (HMA), either Decitabine or Azacytidine, ± Donor Lymphocytes Infu-

sions (DLI). Ten/17 cases (59%) had an unfavorable karyotype at diagnosis and 2/17 (12%) cases were FLT3-ITD positive. Median age was 51 years (range 24-66), 11 were male and 6 were female; 3/17 patients underwent Allo-SCT from an HLA-identical sibling donor, 12/17 from a Matched Unrelated Donor (MUD) and 2/17 from an Haploidentical donor. The median time from transplant to relapse was 6,3 months (range 2,3-72,5), 16/17 had a cytologic relapse (with an average blast count of 40%), while 1/17 had a molecular relapse. Eleven/17 patients (65%) received salvage therapy after Allo-SCT with Azacytidine while 6/17 (35%) received Decitabine. Eight/17 cases (47%) received concomitant HMA and Donor Lymphocytes Infusions (DLI) according to DLI availability. Patients received a median of 2 cycles of HMA therapy (range 1-9) and a median of 3 DLI doses (range 1 - 9). After a median follow-up of 3 months (range 1–38) from the beginning of the HMA therapy, 12/17 (71%) patients died, of these 8/12 (67%) due to AML and 4/12 (33%) due to transplant related mortality. Five/12 patients (29%) are alive, of these 1/5 (20%) in complete remission (CR) and 4/5 with active, but stable, disease. No patient experienced a GVHD after DLI. The median OS of this population was 7 months with a 1 year OS probability of 10%. We observed a significant survival improvement in patients that received concomitant DLI (median OS 10 months *vs* 2,2 months, P=0,0001-Figure 1) and in patients that achieved a CR with HMA (median OS 12,3 months *vs* 3 months, P=0,017). *Conclusions:* Our real life experience suggests that HMA as salvage therapy is feasible and safe. We observe a clear OS benefit only in patients who received a combination of HMA plus DLI suggesting a synergistic effect. Studies are needed to explore the benefit of HMA combinations with DLI and/or new drugs as salvage therapy for AML after Allo-SCT or, even better, as prophylactic or pre-emptive strategy to prevent cytologic relapse after Allo-SCT.

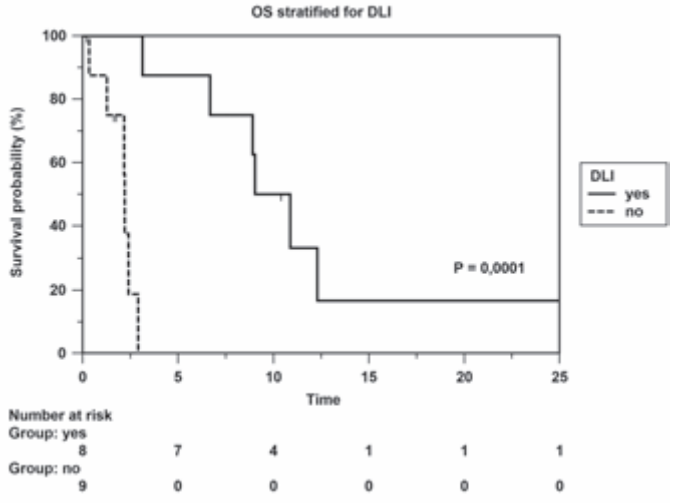

Figure 1.

P047

PERFORMANCE AND TOXICITY OF FLAI (FLUDARABINE, CYTARABINE, IDARUBICINE) AS INDUCTION CHEMOTHERAPY FOR HIGH RISK SECONDARY ACUTE MYELOID LEUKEMIA ARISING FROM PHILADELPHIA-NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS OR MYELODYSPLASTIC SYNDROMES

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Introduction: The aim of this study was to evaluate the outcome and the safety profile of FLAI as induction chemotherapy regimen in high risk AML, secondary to Myelodysplastic Syndromes (MDS) or Ph negative Chronic Myeloproliferative Neoplasms (MPN). The rationale of FLAI combination is that Fludarabine is a fluorinated purine analogue that is toxic against Multidrug Resistance (MDR) overexpression cells and enhances Ara-C cytotoxicity by increasing cellular concentration of Ara-C 5 triphosphate, thus inhibiting DNA repair. *Patients and Results:* 72 consecutive high risk AML arising from Ph neg MPD (15/72, 21%) or MDS (57/72, 79%) were treated with FLAI induction regimen at our Dpt in the last 10 years. The M/F ratio was 35/37 with a median age of 63 yrs (range 24-75). The MDR (PGP > 6 MFI) phenotype was present in 33/72 cases (46%). The induction regimen (FLAI) included: Fludarabine (25 mg/sqm), Ara-C (2 g/sqm) on days 1-4, Idarubicin (8-10 mg/sqm) on days 1-3. Pts were evaluated for response rate, treatment-related adverse events, Overall Survival (OS) and Disease Free Survival (DFS). After FLAI, complete remission (CR) occurred in 60% of pts (42 of 70 evaluable cases); 8/70 pts (11%) achieved a PR and 20/70 (29%) were resistant. There was 2 cases of death during induction (DDI 3%). Proven infections occurred in 39/72 pts (54%), including BSI (25 episodes) and/or pneumonia (14 cases). Grade II-III WHO oral mucositis was reported in 13 pts (18%). Median time to neutrophil (> 1x109/l) and platelet (> 50x109/l) recovery was 22 (range 19-42) and 26 (range 20-43) days, respectively. Supportive therapy consisted of a median of 24 RBC (range 4-42) and 8 platelets units (range 2-49). G-CSF was administered in 20/72 (28%) pts. The biologic and clinical characteristics at diagnosis and their relationship with CR achievement, OS and DFS will be analyzed and reported. Overall, after a median follow-up of 19 months (range 1-101), 30/72 (42%) pts are alive (27/30 in CR). The probability of 1-year OS and DFS were 65 and 59%. *Conclusions:* The FLAI induction regimen, as an example of conventional chemotherapy, is effective in secondary AML arising from MDS or Ph neg MPN with a favorable CR rate, acceptable safety profile and low DDI. Therefore, despite the development of new drugs, FLAI remains a valid option, in clinical practice, such as induction CHT in secondary AML and could be considered a good candidate as a control arm in randomized clinical trials with new drugs.

P048

THE FORKHEAD BOX C1 (FOXC1) TRANSCRIPTION FACTOR IS DOWNREGULATED IN ACUTE PROMYELOCYTIC LEUKEMIA

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Forkhead box (FOX) genes, encoding transcription factor regulators of the embryogenesis, are involved in cell differentiation and in the maintenance of the mesenchymal niche in the hematopoietic system. Overexpression of FOXC1 has been reported in solid tumors and AML. We found that FOXC1 mRNA and protein levels were significantly lower in primary marrow samples from 27 APL, as compared to 27 patients with other AML subtypes, and 5 normal CD34+ cells. FOXC1 expression significantly increased in marrow samples collected from APL at the time of remission following consolidation treatment. In keeping with the observations made in diagnostic samples, NB4 t(15;17)-positive cells exhibited low FOXC1 expression levels, which increased following ATRA treatment, whereas in the NB4-R4, a derivative of NB4 resistant to ATRA due to a point mutation in the RA binding region of PML/RARA, FOXC1 levels remained stable after ATRA treatment. Induction of PML/RARA by Zn2+ addition in MTPR9 cell line, a derivative of U937 expressing PML/RARA under the control of a Zn2+-inducible promoter, downmodulated FOXC1 expression at both mRNA and protein levels. Similarly, transfection of PML/RARA in the human embryonic kidney 293 (HEK) cell line markedly decreased FOXC1 levels. Downregulation of FOXC1 was associated to binding of PML/RARA to the FOXC1 promoter region spanning 398 to 391, studied by a ChIP assay using nuclear extracts from the NB4 cell line. Addition of ATRA decreased PML/RARA binding and induced upregulation of its expression in NB4 cells. Moreover, reduced FOXC1 expression was consistently associated to DNA hypermethylation of the +354 to +568 FOXC1 region. The FOXC1 methylation pattern in samples collected after consolidation treatment was similar to that observed in normal BM. FOXC1 methylation was higher in NB4 than in other cell lines, and did not change either upon ATRA treatment or after induction of PML/RARA expression both in MTPR9 and HEK cell lines. FOXC1 methylation was functional and modular, since treatment of the HL-60

(myeloblastic cell line) and NB4 cell lines with decitabine induced FOXC1 demethylation and up-regulated FOXC1 expression. Of note, decitabine treatment induced FOXC1 demethylation and up-regulation also in ATRA-resistant NB4-R4 cells. Our findings indicate that FOXC1 is repressed in APL due to hypermethylation and to the presence of the PML-RARA rearrangement, and suggest a potential role of hypomethylating treatment in this disease.

P049

BIOMARKER BASED POST-REMISSION STRATEGY IMPROVES THE OUTCOME OF ADULT AML PATIENTS

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In adult acute myeloid leukemia (AML), measurement of minimal residual disease (MRD) in the remission phase is increasingly used, together with the baseline cytogenetic/molecular signature, for a more appropriate outcome prediction and post-remission therapy selection. The aim of our study was to evaluate if a strategy of biomarker-based, risk-adapted post-remission therapy will translate into an improved overall survival (OS) and disease free survival (DFS). We designed a risk-adapted strategy (RA-Tx) in which high-risk (adverse karyotype, FLT3-ITD mutations) and low risk (CBF-AML and NPM1 mutated) patients should receive allogeneic stem cell transplantation (ASCT) or autologous SCT (AuSCT). For patients belonging to the intermediate risk category, we designed a biomarker-based strategy (BB-Tx) in which SCT or AuSCT was delivered depending on the MRD status at the end of consolidation. Definition of MRD positivity required ≥ 3.5x10-4 residual leukemic to be counted in the bone marrow upon full hematological recovery following post consolidation cycle. For comparison, we analyzed the outcome of a matched historical cohort of patients who were submitted to ASCT if a fully matched family donor was available or to AuSCT (if lack of a donor), without taking into account the cytogenetic/genetic signature at diagnosis or the MRD status (standard treatment, SD-Tx). Among 175 evaluable patients, 52 (30%) belonged to the high-risk, 68 (39%) to the intermediate-risk and 55 (31%) to the low-risk category, respectively. One hundred-17 (67%) received RA-TX/BB-Tx and 58 (33%) SD-Tx. At 10-years, in the whole series OS (45.3% *vs* 27.6%, p=0.01) and DFS (36.2% *vs* 24.1%, p=0.05) were longer for RA-TX/BB-Tx as compared to SD-Tx. Focusing on the different risk subgroups, we observed that in high- and intermediate-risk pts, OS and DFS were longer for those in RA-TX/BB-Tx. However, among patients in the low risk-category, those receiving SD-Tx had a non-significant, superior outcome. A possible explanation is that in the low-risk group a relevant proportion of pts was MRD positive so that AuSCT might have not been as effective as expected in providing a long-term disease control. A program of biomarker-based, risk-adapted post consolidation treatment selection is feasible. In the high-risk and intermediate MRD positive category, ASCT ameliorates prognosis. In low-risk patients, AuSCT might not be sufficient to control leukemia outgrowth if MRD negativity is not achieved.

P050

MULTILINEAGE DYSPLASIA AS ASSESSED BY IMMUNOPHENOTYPE IN ACUTE MYELOID LEUKEMIA: A PROGNOSTIC TOOL IN GENETICALLY UNDEFINED CATEGORY

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Background: Acute myeloid leukemia (AML) "with myelodysplasia-related changes" is classified separately by WHO 2016. Its actual prognostic significance is debated due to technical and biological reasons. Technical ones deal with standard criteria for defining MLD, that is morphology, operator-dependent and challenging at AML diagnosis. Biologically, MLD-related unfavourable prognosis would rely on preceding clonal hemopoiesis but it might merely result from belonging to AML clone. In defined genetic subsets (NPM1- or bi-allelic CEBPA-mutated) MLD role has been downsized. In less characterized groups, MLD significance is discussed as potential support for major decisions. Aim. To study MLD by flow cytometry (FC), alternative to morphology and emerging as a method to study dysplasia, by investigating key antigens throughout maturation. *Methods:* Patients entering the study had newly diagnosed AML and were intensively treated. Karyotype and search for mutations in NPM1, FLT3 and CEBPA were carried out according to published evidences. FC: Dysplasia was appraised for neutrophils and erythroid cells by an immunophenotypic score (IPS) including 17 parameters (13 for neutrophil and 4 for erythroid). Controls were used to set normal phenotypic profile. A score was calculated for each parameter based on the extent of deviation from normal ranges. *Results:* Since 2005 to 2014, 231 pts were studied and classified according to IPS-defined dysplasia. Consistent with WHO-defined MLD, based on involvement of multiple lineages, 156 (67.5%) pts were defined dysplastic (IPS_LIN+) because both lineages were dysplastic by IPS. Conversely, 75 (32.5%) pts were defined IPS_LIN-. No major differences emerged regarding main disease characteristics. As per outcome, overall CR rate was 74.0%. No difference in CR rate was observed: 72.4% and 77.3% in IPS_LIN- and IPS_LIN+, respectively (p=0.52). IPS did not affect survival in the overall cohort (Figure 1A-B) nor focusing on intermediate or adverse karyotype categories. In "triple-negative" pts (n=54), lacking meaningful genetic abnormalities (normal karyotype, no mutations for NPM1, FLT3 and CEBPA), IPS showed a significant impact on overall survival (Figure 1C). By splitting pts for number of dysplastic lineages, we observed a prognostic gradient with no-dysplastic-lineages group showing the best outcome (Figure 1D). *Conclusions:* FC-assessed MLD might aid prognostic stratification of undefined AML potentially guiding treatment strategy.

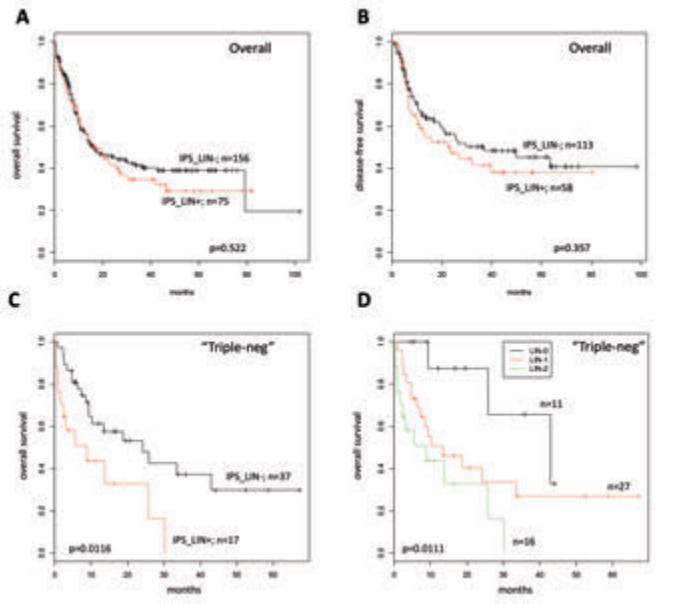

Figure 1.

P051

COEXISTING MUTATIONS IN NPM1 MUTATED ACUTE MYELOID LEUKEMIA

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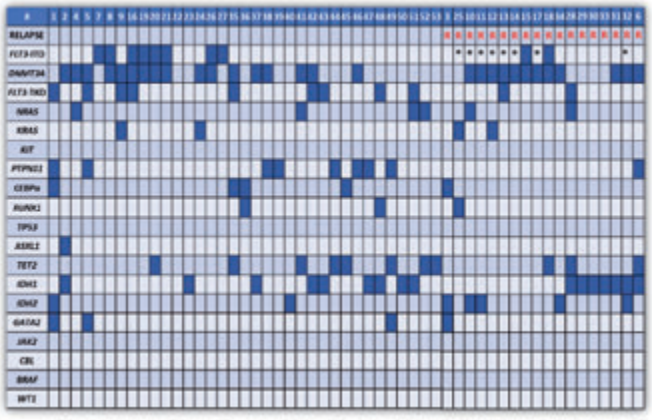
NPM1 mutations represent a frequent genetic alteration in acute myeloid leukemia (AML), occurring in approximately 30% of patients and more frequently in cases with normal karyotype (NK-AML). They are independently associated with favorable outcome. Recent studies using Next Generation Sequencing (NGS), have allowed the identification of several novel mutations in different cellular pathways. This may also allow dissection of the heterogeneous group of NK-AML into prog-

nostically different subgroups. Here, we studied the mutational landscape in a cohort of NK NPM1(mut) *de novo* AML patients, using the Ion Torrent (TM) NGS System, coupled with the Ion AmpliSeq(TM) technology. DNA isolated at diagnosis from 53 NPM1(mut) AML patients was sequenced for the following 19 genes, known to be commonly mutated in myeloid malignancies: NPM1, DNMT3A, FLT3-TKD, NRAS, KRAS, KIT, PTPN11, CEBP, RUNX1, TP53, ASXL1, TET2, IDH1, IDH2, GATA2, JAK2, CBL, BRAF and WT1. Sequencing data were analysed using the Ion Reporter(TM) Software (Thermo Fisher Scientific), using the 5% threshold. Mutations were then validated by Sanger sequencing. The mean number of additional variants was 4.69 per patient, to a total of 61 known variants in 13 genes. NPM1 mutations were commonly associated with mutations in DNMT3A (54%), IDH1(R132) (30%), and FLT3 (51%) genes, while none of the cases harbored mutations in cKIT, TP53, JAK2, CBL, WT1 and BRAF genes (Table 1). Eighteen patients relapsed, while 35 were in continuous complete remission at the time of analysis. Eight of 18 patients who relapsed, were retrospectively found to carry a subclonal FLT3-ITD mutation at diagnosis. Looking at associations between the most frequent mutations, we found that all DNMT3A (mut) patients were also NPM1(mut) (n=29). The subset of double mutated IDH1(mut)/DNMT3A (mut) identified a patient subgroup with unfavorable outcome, with a significantly higher incidence of relapse (7/12, 57% relapsed patients *vs* 3/17, 18% patient remaining in CCR, p= 0.046). Other associations of mutations of NPM1, DNMT3A, FLT3-ITD at diagnosis were not predictive of relapse. In conclusion, the presence of FLT3-ITD+ subclones at diagnosis identifies prognostically unfavorable NPM1(mut) patients, similar to IDH1 mutations in DNMT3A (mut) AML, who may benefit from treatment intensification at time of remission.

Table 1. Mutational landscape in 53 *de novo* NPM1-mutated AML. Each column represents a patient. Mutated samples are indicated in blue. R: Relapse *:FLT3-ITD subclones.



P052

KNOCKDOWN OF MIR-128A INDUCES LIN28A EXPRESSION AND REVERTS MYELOID DIFFERENTIATION BLOCKAGE IN ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) is a heterogeneous hematopoietic stem cell malignancy, characterized by rapid growth and impaired differentiation of neoplastic cells with their abnormal accumulation in bone marrow and peripheral blood. Lin28A is a highly conserved RNA-binding protein that concurs to control the balance between stemness and differentiation in several tissue lineages and plays an important role in cancer stem cells. Its knockdown in mouse hematopoietic system leads to myeloid cells expansion and decrease of B cell number, thus triggering an alteration of hematopoiesis. It has been also reported the existence of reciprocal regulatory loops between Lin28 and its negative regulator miR-128. In particular, this microRNA is expressed in early hematopoietic progenitor cells, preventing the differentiation of all lineages. Moreover, increased expression of mir-128 was associated with high-risk molecular features of AML. Thus, since Lin28 and miR-128, seem to be important regulators of hematopoiesis, it could be interesting to study their involvement in induction and maintenance of an early differentiation status in AML. In our study, using qRT PCR and cytometric analysis, we found Lin28A under-expressed in blast cells from 38 AML patients at diagnosis and 7 AML cell lines respect to CD34+ normal precursors, without any significant association with genotypic and phenotypic features. *In vitro* transfection of Lin28A in NPM1-mutated OCI-AML3 cell line significantly triggered cell cycle arrest and myeloid differentiation, with increased expression of macrophage associate genes (EGR2, ZFP36 and ANXA1). Furthermore, miR-128 was found over-expressed in AML cells compared to normal precursors, especially in acute promyelocytic leukemia (APL) and in "AML with maturation" harboring FLT3 mutation (according to 2016 WHO classification of myeloid neoplasms and acute leukemia). Its forced overexpression by lentiviral infection in OCI-AML3 down-regulated Lin28A with ensuing repression of macrophage-oriented differentiation. Finally, knockdown of miR-128a in OCI-AML3 and in APL/AML leukemic cells (by transfection and lentiviral infection, respectively) induced myeloid cell differentiation and increased expression of Lin28A, EGR2, ZFP36 and ANXA1, reverting myeloid differentiation blockage. In conclusion, our findings revealed a new possible mechanism for AML differentiation blockage, suggesting novel potential therapeutic strategies based upon miR-128a inhibition.

P053

DROPLET DIGITAL PCR IS A RELIABLE TOOL FOR MONITORING MINIMAL RESIDUAL DISEASE IN ACUTE PROMYELOCYTIC LEUKAEMIA

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Nested PCR (nPCR) and Real-Time quantitative PCR (qPCR) are well-established methods for monitoring minimal residual disease (MRD) in Acute Promyelocytic Leukemia (APL). Despite their remarkable sensitivity and specificity, both methods have inherent limitations, such as qualitative MRD evaluation and relative quantification. Here, we employed droplet digital PCR (ddPCR) to monitor MRD in 21 APL patients and compared its performance with nPCR and qPCR. After assessing the Limit of Detection (LOD) for each technique on serial dilutions of PML-RARA bcr1 and bcr3 transcripts, a total of 48 follow-up samples were analysed and the results compared. ddPCR showed good linearity and efficiency and reached a LOD comparable or even superior to nPCR and qPCR. When tested on primary samples, ddPCR sensitivity and specificity were ≥95% and ≥91% for bcr1 and bcr3 transcripts and displayed a significant concordance with both techniques, particularly with nPCR. The peculiar advantage of ddPCR-based monitoring of MRD is represented by absolute quantification, which provides crucial information for the management of patients whose MRD fluctuates under the LOD of qPCR and is detectable but not quantifiable by nPCR. Our findings highlight ddPCR as a reliable complementary approach to monitor MRD in APL, and suggest its advantageous application particularly for the molecular follow-up of patients at high risk of relapse.

P054

MRD DRIVEN CHOICE OF CONSOLIDATION AND MODULATION OF INDUCTION AND CONSOLIDATION INTENSITY RESULTED IN A SIGNIFICANTLY IMPROVED OUTCOME OF YOUNGER AML PATIENTS IN THE LAST THREE YEARS

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In the last decades no new drugs have been introduced for AML. The MRC group has however reported a progressive increase of cure rates

in younger patients. The outcome of younger (<65 years) AML patient treated in our Center has been evaluated in order to disclose if has improved in four consecutive 3-year periods of treatment (from 2005 to 2016) and to recognize factors possibly leading to this result. We reviewed the outcome of 145 consecutive AML patients aged 65 or less who received fludarabine-containing induction followed by high dose Ara-C consolidation or allogeneic stem cell transplant (ASCT). Minimal residual disease (MRD) assessment was performed in all patients, with flow cytometry, WT1 expression levels and, if feasible, with recurrent abnormalities. Patients treated in the last 3 years showed a significant survival improvement (Fig 1), in comparison with the other considered periods. The cohorts of patients treated in the four periods had a comparable age and risk distribution. Beside classical risk factors, the time from recovery after induction I and the start of induction II was related to survival probability, with an optimal interval between 15 and 20 days. Patients treated in the last 3 years had a median time from recovery after cycle 1 to cycle 2 of 17 days, compared to 22 days in the other cohorts ($p<0.05$). After 2013, MRD information was added as a prognostic factor. ELN non-high risk patient with negative MRD after induction I did not receive ASCT, but received an higher dose of Ara-C as consolidation. Among 8 INT-risk patients who were MRD neg and did not proceed to ASCT in CR1, only one relapsed. Conversely, among 5 INT-risk patients who underwent ASCT in CR1 because of MRD positivity, no relapses have been observed. Starting from 2014, patient in first CR who showed MRD reoccurrence underwent salvage therapy before hematologic relapse, followed by ASCT consolidation. MRD-directed therapy allowed all treated patient to achieve MRD neg remission before ASCT. Finally, after 2013, a reduced incidence of invasive fungal infections (IFI) was observed, mainly due to the introduction of prophylaxis with posaconazole. Our experience shows that, even without the contribution of new drugs, more appropriate utilization of ASCT, tailored on early MRD assessment, modulation of chemotherapy intensity guided by a careful evaluation of age and clinical condition, and posaconazole prophylaxis of IFI led to a relevant improvement of outcome.

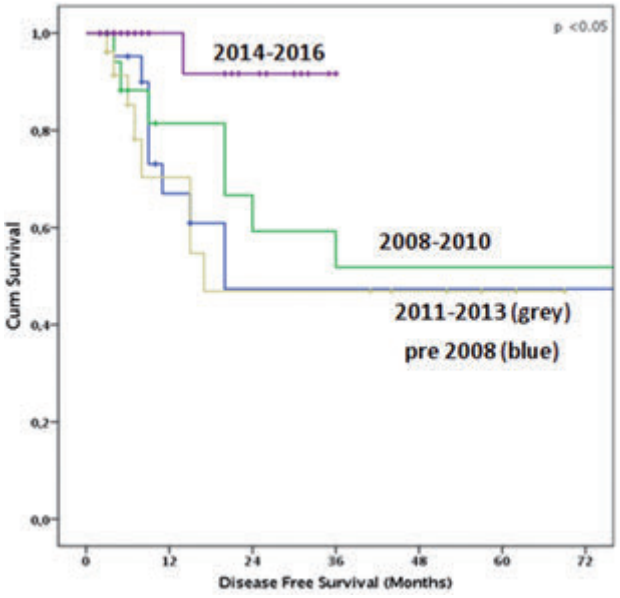

Figure 1.

P055

EXPLOITING THE DNA DAMAGE RESPONSE (DDR) PATHWAY USING AZD-1775, A WEE1 INHIBITOR, TO SENSITIZE ACUTE LYMPHOBLASTIC LEUKEMIA CELLS TO DIFFERENT ANTINEOPLASTIC DRUGS

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Nowadays the number of therapeutic options for the treatment of adult acute lymphoblastic leukemia (ALL) patients is still very poor and, especially for relapsed patients, still based on conventional chemotherapy. Thus, there is a need to improve therapeutic approaches. The inhibition of the DNA damage response (DDR) pathway has extensively being evaluated in preclinical and clinical trials as an innovative strategy to enhance the effectiveness of antineoplastic drugs. Recently different compounds have been screened to selectively inhibit the G2/M checkpoint regulator, the tyrosine/threonine kinase WEE1 which is fundamental to promote cell-cycle arrest. Although inactivating mutations of WEE1 are rare in tumors, the level of expression of this kinase has been found altered in different neoplasms. The aim of the project was to evaluate the effectiveness of AZD-1775 (WEE1 inhibitor) as an innovative strategy for the treatment of ALL. In this study we reported that WEE1 was highly expressed in adult primary ALL samples (n=79) compared to normal mononuclear cells ($p=0.01$). The highest level of expression of WEE1 was found in Ph-positive/-negatives relapsed samples. On these bases we then evaluated the efficacy of AZD-1775 as single agent and in combination with different drugs and we elucidated its mechanisms of action. AZD-1775 reduced cell viability of leukemic cells by forcing the G2/M checkpoint and inducing apoptosis in both B-cell and T-cell precursor ALL cell lines (B/T-ALL) and human primary leukemic blasts (n=15). Moreover, we showed that in our cohort of primary leukemic blasts the sensitivity to AZD-1775 correlated with the expression of the WEE1-family protein, MYT1 (PKMYT1) ($p=0.004$) but not with WEE1. Microscopy analyses showed that AZD-1775 pushed leukemic cells to enter mitosis which ended in mitotic catastrophe, as showed by the induction of micronuclei and DNA bridges. AZD-1775 significantly enhanced the efficacy of tyrosine kinase inhibitor and of different chemotherapeutic agents (clofarabine, doxorubicin and methotrexate) in terms of reduction of cell viability, induction of apoptosis and inhibition of proliferation rate on both primary cells and leukemic cell lines. These data lay the basis for a better evaluation of the therapeutic potential of AZD-1775 as chemo sensitizer agent for the treatment of adult B-/T-ALL. Acknowledgements ELN, AIL, AIRC, PRIN, progetto Regione-Università 2010-12, FP7 NGS-PTL project, HARMONY project.

P056

APOPTOTIC AND DIFFERENTIATING EFFECTS OF THE POLY(ADP-RIBOSE) POLYMERASE INHIBITOR OLAPARIB IN ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROMES

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Background: Olaparib (Lynparza; AstraZeneca) is a potent orally bioavailable poly(ADP-ribose) polymerase inhibitor (PARPi) approved for BRCA1/2-mutated ovarian cancer which is defective in Homologous Recombination repair of DNA double strand breaks. We have recently shown that olaparib exerts anti-proliferative and apoptotic effects in human Acute Myeloid Leukemia (AML) blasts at clinically achievable concentrations, which do not affect the viability of normal bone marrow (BM) stem cells (Faraoni *et al.* Biochim Biophys Acta 2015). The low BRCA1/2 expression levels detected in AML blasts might account for leukemia sensitivity to synthetic lethality induced by olaparib. Besides its involvement in DNA repair, PARP1 also has a crucial role in regulation of gene transcription. Since transcription deregulation is common in hematopoietic malignancies, we analyzed whether modulation of gene expression by PARPi may contribute to the anti-leukemia activity of olaparib. Moreover, we investigated whether olaparib induces differentiation in myelodysplastic syndromes (MDS), which are characterized by ineffective hematopoiesis due to the aberrant activity of oncogenic transcription factors. *Results:* Mononuclear cells from 20 AML and 20 MDS adult patients were isolated from BM aspirates and treated *in vitro* with graded concentrations of olaparib (1.25-10 μM). The results indicated that human AML blasts express lower levels of the death receptors FAS (CD95) (unpaired Stu-

dent's t-test, p<0.001) and DR5 (TRAIL receptor) (p<0.01) transcripts compared to BM from healthy donors. Interestingly, apoptosis triggered by olaparib is associated with a dose-dependent up-regulation of death receptor transcripts (~3-fold increase at 10μM, FAS p<0.01; DR5 p<0.05) that requires NF- B activation. In MDS olaparib induces apoptosis and, in surviving cells, it induces myeloid differentiation, as shown by the increase of cell populations expressing markers of neutrophils (CD11b+/CD16+ or CD10+/CD15+) and monocytes (CD33+/CD64+). The PARPi also increases the expression of PU.1 (~3-fold increase at 10 μM, p<0.01) and CEBPA (~2-fold, p<0.05), which are transcription factors involved in the maturation of hematopoietic myeloid cells. *Conclusion:* Our results suggest that in addition to the mechanism of synthetic lethality, olaparib induces cell death in primary AML blasts stimulating FAS and DR5 death receptor expression and cell differentiation in MDS.

P057

INCIDENCE, EPIDEMIOLOGY AND MICROBIOLOGIC SPECTRUM OF FEBRILE NEUTROPENIA IN ACUTE MYELOID LEUKEMIA: A RETROSPECTIVE ANALYSIS IN 1048 NEUTROPENIC FEBRILLE EPISODES IN A SINGLE CENTRE

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Neutropenic fever determines morbidity and mortality during intensive chemotherapy induced neutropenia in adult acute myeloid leukemia (AML) patients. Prophylactic and empiric treatment, based on international recommendation at the time, could vary among the centers, according to local epidemiology. We retrospectively analyzed a total of 1048 neutropenic episodes after induction and consolidation chemotherapy cycles in order to determine the potential etiology, microbiologic spectrum, response and resistance to antibiotics together with the patients outcome. A total of 334 consecutive *de novo* AML patients with a median age of 53 years, ranging from 18-76 years, were treated with intensive chemotherapy in a single hematologic institution. Complete remission was achieved in 238 patients (71%), 74 patients were chemotherapy resistant (22%) and 22 patients died, marking an induction mortality of 7%. A subgroup of 112 patients (34%) achieving a complete remission consequently underwent ASCT. Further, incidence, etiology and clinical complications of neutropenic fever periods during the whole treatment have been studied. In 418 cycles no fever was observed. A fever of unknown origin (FUO) occurred during 230 neutropenia periods whereas a clinically documented infection was present in 106 aplasia periods. A microbiologically documented infection was evident in 272 cases and an invasive fungal infection (IFI) present after 22 cycles. A statistically significant difference was documented analyzing the microbiological spectrum of the infections, incidence of Gram+ bacteria were significantly more evident during the first induction cycle compared to Gram– bacteria, that were significantly more isolated after consolidation treatments (66/119 *vs* 67/93), p=0.001. In conclusion in the present large and homogeneous cohort of AML patients the pattern of infections changed during the ongoing chemotherapy treatment. This may be attributed to the prophylactic and therapeutic use of antibiotics, that may select the microflora. Evaluation of antibacterial spectrum and resistance patterns in an institution must be done routinely in order to choose empiric antibiotics therapy. Based on the results careful selection of adaptive antibiotic regimens may help in reducing morbidity and mortality during AML therapy.

P058

BLINATUMOMAB IS SAFE AND EFFECTIVE IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: A SINGLE CENTER EXPERIENCE

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Introduction: Patients with relapsed/refractory (R/R) acute lymphoblastic leukemia (ALL) have a dismal prognosis with current chemotherapy regimens. Similarly, persistence of molecular minimal residual disease (MRD) indicates resistance to conventional chemotherapy and needs for new approaches to prevent clinical relapse. Anti-CD19 Bispecific T Cell- Engager Blinatumomab (Blina) showed hematological and molecular response in these settings. We report our single center experience on R/R or MRD-positive ALL treated with Blina, aiming to assess its tolerability and efficacy. *Methods:* From February 2014 to March 2016, 25 adult ALL patients (19 R/R and 6 MRD-positive) were treated at our Institute with Blina, 4-weeks continuous intravenous infusion at fixed stepwise doses (9 μg/day in week 1 of cycle 1, followed by 28 μg/day thereafter), followed by a 2-week treatment-free interval. 14 patients were BCR-ABL1 positive. Median age was 42 years (range 18-73), with 8 patients above 60 years. The median number of previous therapies was 3 (range 1-7); 11/14 Ph+ ALL had received third generation TKI. Six patients had already received allogeneic stem cell transplantation (allo-SCT). *Results:* 13/25 patients obtained a complete remission (CR), with 11/13 molecular CR (mCR). Three patients had a stable disease and 7 a disease progression. One patient died due to infectious complication during the first cycle and one was not evaluable, not completing first cycle. The median number of cycles to response was 1. Four MRD-negative patients relapsed while on Blina therapy, one of which with extramedullary disease, and 6 underwent allo-SCT while in mCR. With a median follow-up of 11 months (range 1-38) 10/25 (40%) patients are alive in CR (7 after allo-SCT). 15 patients died, 1 in mCR due to SCT complications, 1 for infection during first Blina cycle, and 13 for R/R ALL. The most common non hematological toxicity was fever (19/25) with microbiological documentation in 4 cases; two patients had grade III neurological events, one (mental confusion) requiring permanent treatment interruption. Grade III cytokine release syndrome was observed in 1 case. *Conclusions:* Single agent Blina confirmed its antileukemia activity in heavily treated ALL patients, including Ph+ ones, being safe even in an elderly population otherwise without any therapeutic chance. Acknowledgements BolognAIL, ELN, AIRC, PRIN, Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project, HARMONY project.

P059

CYTOGENETIC AND MOLECULAR RISK FACTORS AT DIAGNOSIS ARE ABROGATED BY WT1 AND FLOW CYTOMETRY-BASED PRE TRANSPLANT MINIMAL RESIDUAL DISEASE ASSESSMENT IN ADVANCED ACUTE MYELOID LEUKEMIA PATIENTS

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Allogeneic bone marrow transplantation (BMT) offers the only chance of cure for patients with advanced acute myeloid leukemia (AML). High levels of pre BMT minimal residual disease (MRD) have been reported to predict relapse risk after BMT even in patient transplanted in first complete remission (CR). WT1 expression levels and multicolor flow cytometry (MFC) are the most common tools to evaluate MRD. The aim of this study was to analyze the role of pre-BMT MRD assessment as predictor for the post-transplant relapse risk in advanced AML patients. We retrospectively analyzed the outcome of 92 consecutive AML patients receiving allo-BMT in 2nd or 3rd CR. Pre-BMT bone marrow (BM) samples were analysed for WT1 expression and MFC as MRD evaluation. Median age was 45 years. Disease phase was CR2 in 63 (68%) and CR3 in 29 patients (32%). ELN risk at diagnosis was low in 28 (30%), intermediate in 44 (48%) and high in 20 (22%) patients. Median follow-up was 64 months. MFC MRD was performed at four or eight (since 2011) color flow-cytometry. WT1 copy number/Abl copy number 250x104 was used as cut-off value for abnormal WT1 expression. Relapse occurred in 30 patients (33%) and two years non-relapse mortality was 29%. Three-year estimate of OS was 47.9% (median 19 months). The survival probability was significantly affected by donor source (better for HAPLO, p<0.05), ELN risk at diagnosis (better for ELN low risk, p<0.01), MRD status before BMT measured with any method (p <0.01 for WT1-based MRD, p<0.03 for MFC based MRD) and CR status at BMT (better for CR2, p<0.05). Specifically, among MRD negative patient a relatively low rate of relapse was observed regardless of risk at diagnosis (2-years OS of 62.2% and

52.7% among MFC MRD negative patient with ELN risk low or intermediate/high, respectively, Figure 1). Multivariate OS analysis revealed that the MRD evaluation by any method was the only independent predictor of OS (p <0.05 for both). Pre transplant MRD evaluation by both WT1 and MFC on BM samples is a reliable predictor of relapse risk which may overcome the prediction based on ELN risk at diagnosis. Patients with positive MRD markers display an higher risk of relapse, irrespectively of having a low ELN risk at diagnosis. Refining the baseline risk assessment in patient undergoing BMT beyond CR1 is crucial and could allow to apply pre-emptive therapeutic strategies to prevent AML relapse, from donor lymphocyte infusion to other innovative approaches.

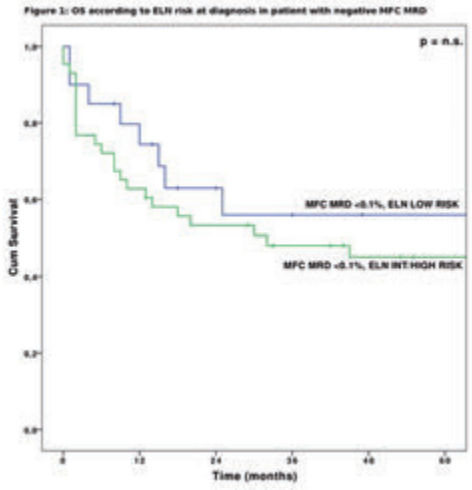

Figure 1.

P060

ACUTE PROMYELOCYTIC LEUKEMIA (APL) IN ELDERLY PATIENTS IS NOT RARE, EVEN OVER THE AGE OF 70, AND CAN BE SUCCESSFULLY MANAGED BY STANDARD TREATMENTS AT ADAPTED DOSES. A SINGLE CENTER SERIES OF 39 PATIENTS

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APL is considered relatively rare in older age. A higher early death rate, comorbidities and poor clinical conditions may account for the low incidence of elderly patients (pts) in clinical studies. So the best therapeutic strategy in elderly APL is still unclear, particularly for pts aged >70. We report an analysis of the outcome of the consecutive series of pts with APL aged >60 diagnosed at our Institution between 2000 and 2016. Among 95 pts with APL confirmed by PML-RARalfa detection, 39 (37%) were aged over 60 (range 61-89 y, median 70) of which 21 >70 (20%) who represent the largest single Center series reported so far. Treatment was modulated by physician's decision, considering APL risk (PETHEMA-GIMEMA score), drug availability, comorbidities and fitness. Induction consisted of ATRA monotherapy in 6/39, ATRA+idarubicin at adjusted doses in 26/39, or ATO+ATRA in 7/39 pts. Consolidation and maintenance were also modulated. Two pts <69 and 11/20 >70y were unfit, according to Ferrara (Leukemia, 2013). Hematological complete remission (CR) rate was 97,4%, molecular CR rate 92,3%, with no impact of type or intensity of induction. One pt, receiving ATRA only, died early of cerebral hemorrhage. Side effects included infections (11), differentiation syndrome (14), cardiovascular events (8), with two myocardial infarctions, but were successfully managed. Infectious complications occurred also post-induction [herpes-zoster(1), recurrent urinary tract infections (1), endocarditis (1), FUO (2) and aspergillosis (1)]. The median observation period was 48 months. Survival at 5-y was 67%; it significantly differed according to induction and age, being 100% in 7 pts aged <70 receiving ATO/ATRA, 74% in pts receiving ATRA+chemotherapy, and in 44% pts receiving ATRA only (P=0.023; Figure1A). In pts >70 the outcome was significantly better when idarubicin was included in induction (65% *vs* 15% P=0.025; Fig 1B). APL relapsed in 6 pts (15.8%), all aged >70, without relation to APL risk, or idarubicin dose. Thirteen pts died, of active APL (4), treatment-related complications (3), or unrelated comorbidities (6). We conclude that, as in population-based registry studies (Lehmann, 2011), nearly 40% of APL are elderly and 20% are older than 70. However current treatment strategies may be successfully adapted to elderly APL obtaining long-term survival similar to younger APL in pts aged 60-69, but remarkable even in pts aged >70 treated with ATRA+IDA.

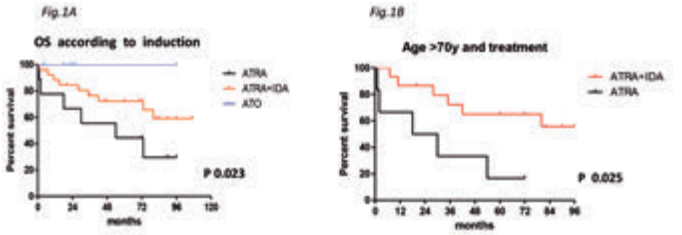

Figure 1.

Acute Leukemias 2

P061

COMPLETE RESPONSE TO BLINATUMOMAB IN AN UNUSUAL EXTRAMEDULLARY RELAPSED B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Extramedullary relapse without bone marrow involvement of acute lymphoblastic leukemia (ALL) in adults is rare and has particular predilection for the central nervous system, lymph nodes, spleen, liver and testis. Blinatumomab is a novel bispecific T cell engager antibody construct that simultaneously binds CD3-positive cytotoxic T cells and CD19 antigen stably expressed on the majority of B-cell ALL blasts. Blinatumomab was first explored in MRD-positive ALL patients and then in relapsed-refractory B-ALL ones, but in extramedullary disease the available experiences are very limited. *Report:* Here we report the case of a 54-years old woman with previous diagnosis of standard risk B common ALL in January 2013 responsive to standard chemotherapy including four-drug induction, consolidation, interim maintenance, delayed intensification and maintenance. Due to the acute onset of metrorrhagia in April 2016 (during the last 6 months of maintenance therapy), a pelvic magnetic resonance was performed, revealing a bulky uterine mass (146x105mm) which required hysterectomy. Histological analysis was consistent with B-cell ALL showing immunohistochemical features overlapping with those of bone marrow at diagnosis. A PET-CT scan showed pathological uptake at left iliac lymph node, left iliac wing, vaginal fornix and left humerus. Bone marrow aspirate showed the absence of leukemic infiltration. The patient was started on a salvage regimen without success: extramedullary disease persisted and bone marrow analysis, whilst confirming hematological remission, showed minimal residual disease (MRD) positivity at flow-cytometry (0.7% blasts infiltration, with technique sensitivity of 10^{-4}). Given the CD19-positivity of the leukemic cells, the patient was enrolled in a compassionate use of blinatumomab. The treatment was well tolerated with only a mild fever (38°C) documented for few days upon steroid withdrawal. After two cycles of immunotherapy, the PET-CT showed complete remission of the extramedullary disease and bone marrow MRD was negative. *Conclusions:* Extramedullary relapse in ALL in adults is uncommon and has a dismal outcome. In particular, the involvement of the uterus, as described in our patient, is extremely rare. Despite the response of the extramedullary disease to blinatumumab needs to be assessed in clinical trials, our report shows that this new therapeutic agent can be highly effective also in such unfavourable conditions.

P062

PATIENT FITTNESS ASSESSMENT AT DIAGNOSIS IN ELDERLY ACUTE MYELOID LEUKEMIA

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Defining the subset of elderly acute myeloid leukemia (AML) patients who are eligible or "fit" for intensive chemotherapy involves a great deal of subjectivity. Criteria yet have to be standardized across or within institutions. Aim of this study was to investigate the validity of four scores for assessment of patient fitness at diagnosis in parallel to physician evaluation. Further patient outcome according the respective evaluation was compared. A total of 85 consecutive elderly (>60 years) patients with newly diagnosed AML were treated. Therapy intensity decision was based on an initial haematologist evaluation followed by discussion of the patient case in an interdisciplinary board. Independently in parallel the local geriatric G8 screening tool, the HCT-CI comorbidity score as well as the AML scores predicting probability of complete remission (CR) and early death (ED) were performed. Overall survival from diagnosis was compared between groups using the Cox model. A total of 42 (49,4%) patients were evaluated "fit" by the medical board and treated by intensive chemotherapy, whereas 4 patients (5%) underwent semi-intensive and 39 patients (45,8%) received palliative therapy. After the first cycle 26 (30,6%) achieved a CR, 44 (51,8%) were non responders and 15 (17,6%) died. Primary physician care evaluation was able to define significantly a "fit" from an "unfit" patient, with a median survival with 10 months (95%CI 5-not reached) compared to 3,4 months (95%CI 1,4-5), p<0.001 with a HR (95%CI) of 3,18 (1,81 to 5,59) in the respective groups. Parallel evaluation of patients unfitness according the proposed G8 (≤14), AML for CR (<40) and AML for ED (≥30) scores discriminated significantly patients survival with HRs equal to 3.03 (p<0,001), 2.11 (p=0,007) and 2.83 (p<0.001), respectively. The agreement between the frailty scores and physician evaluation on the prediction of fitness classification analyzed by calculating the Cohens' Kappa was moderate for HTCI score and AML score for CR (0.47 and 0.46, respectively). The agreement was fair for G8 and AML score for ED (0.27 and 0.33, respectively). In conclusion, in the present AML cohort the applied frailty scores at diagnosis correlated significantly with the median overall survival. Since no perfect agreement was found respect to physician for fitness classification, frailty scores can help to improve the prognosis prediction. These results may encourage a following multi-centre analysis.

P063

BITTER TASTE RECEPTORS ARE EXPRESSED ON ACUTE MYELOID LEUKEMIA CELLS AND THEIR STIMULATION MODULATES LEUKEMIA CELL FUNCTIONS

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Acute Myeloid Leukemia (AML) is a clonal disease sprouting from a rare population of leukemic stem cells (LSCs). Over the past years, increasing interest is gaining the contribution that cell-extrinsic factors have in AML generation and maintenance. In this context, the ability of AML cells to detect and sense the changes in the bone marrow (BM) microenvironment is important for different cell functions. Bitter taste receptors (TAS2Rs) are typical G-protein coupled receptors (GPRs) and are normally found on the surface of the tongue, where they facilitate the bitter taste. More recently, their expression has been envisaged in tissues outside the gastrointestinal tract, thus suggesting a wider function in sensing microenvironmental "danger" molecules. So far, very few data about the expression of TAS2Rs in cancer cells are available. In particular, their expression and function in AML cells has not been investigated. In the present work, for the first time we show that leukemia cell lines OCI-AML3, THP-1, KG1 and AML primary cells expressed several T2R subtypes. The expression of TAS2R was associated with typical GPR-related downstream targets, including the beta subunit of gustducin and the PLC beta 2. Stimulation of leukemia cell lines with denatonium, a T2Rs agonist, induced intracellular Ca2+ concentration increase, thus demonstrating T2Rs functionality. GEP analysis identified a number of genes significantly modulated by denatonium treatment. Specifically, leukemic cells stimulated with T2R agonist underwent a down-regulation of genes involved in cell proliferation, cell cycle progression and migration. Functional assays confirmed molecular data. In particular, depending on the extent of stimulation, T2Rs activation inhibited leukemia cell proliferation inducing cell cycle arrest in G0/G1 phase or reduced cell viability inducing apoptosis, as demonstrated by caspase cascade activation and mitochondrial stress induction. Of note, a pronounced inhibitory effect of denatonium on leukemia cells motility, both spontaneous and in response to CXCL-12, was observed. Overall, our data strongly suggest that TAS2R expression represents a novel receptor-based pathway by which AML cells "taste" BM microenvironment. These results may have implications for the discovery of novel functional pathways modulating normal and leukemic hematopoiesis and for the development of a new class of therapeutic molecules.

P064

AZACITIDINE FRONTLINE AND SALVAGE THERAPY FOR ACUTE MYELOID LEUKEMIA PATIENTS. CLINICAL EXPERIENCE AND DEVELOPMENT OF A NEW RISK SCORE

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Acute myeloid leukemia (AML) is an aggressive hematological malignancy, characterized by a poor prognosis. In patients with treatment naive (TN) or relapsed/refractory (R/R) AML therapy with Azacitidine (AZA) has been proven to induce significant response and increased survival in clinical trials. The aim of this study was to evaluate the overall survival (OS) of AZA in patients with TN, not eligible for intensive chemotherapy, and R/R AML and to identify clinical and biological variables associated with increased survival. Twenty-three patients with AML treated with AZA and followed at Padua University Hospital were retrospectively analysed. The diagnosis was made according to 2008 WHO criteria. All patients received s.c. 5-Azacitidine 75 mg/m^2 for 7 days every 4 weeks until disease progression. The median age at diagnosis was 62 years (range 49-81). 16 (70%) patients were TN and 7 (30%) were R/R, with a medium lines of previous therapy of two. Eight patients (35%) were considered at high, 13 (57%) intermediate and 2 (8%) low-cytogenetic risk. Complete remission was documented in 47% patients. The median OS for the whole cohort was 10.7 months, without any difference between TN and R/R patients (p=0.2743). In univariate analysis, variables associated with increased OS were age >70y (p=0.036), no adverse cytogenetic-risk (p=0.021), receiving at least 8 cycles (p<0.0001), reaching a partial remission (p=0.0037), need of ≤ 2 red cell transfusion/cycle (p=0.0062) and ≤ 1 platelets transfusion/cycle (p=0.0011). All these variables were confirmed in multivariate analysis. There were no differences in OS for the percentage of peripheral and bone marrow blast cells. Based on hazard ratios obtained by multivariate analysis we assigned a risk-value to each significant variable. A risk score was then calculated as the sum of each risk value, ranging from 0 to 10. We found that median OS were 16.9 and 6.9 months for patients with ≤4 points and those with 5-10 score, respectively (p<0.0001). We herein provide evidence that 5-Azacitidine is feasible and effective in patients with acute myeloid leukemia, regardless the lines of treatment we are dealing with. Moreover, we developed a scoring system able to identify patients with long lasting response and improved survival.

P065

HYPOMETHYLATING AGENTS IN THE TREATMENT OF UNFIT ACUTE MYELOID LEUKEMIA PATIENTS: A REAL LIFE EXPERIENCE IN A SINGLE CENTER

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Background: Acute Myeloid Leukemia (AML) treatment remains poorly defined in unfit patients (pts). The treatment depends on hematological features, comorbidites and socio-economic conditions. In this subset, hypomethylating agents (HMAs) represents a feasible choise respect to intensive chemotherapy. *Aims:* To evaluate a retrospective analysis on outcome of unfit AML pts not eligible to conventional intensive chemotherapy based on conceptual and operational criteria proposed by consensus of SIE,SIES and GITMO group. *Patients and Methods:* A retrospective analysis was performed in order to evaluate Overall Survival (OS), Overall Respose Rate (ORR) and infectivous complications. From January 2015 to April 2017 a total of 55 AML pts were diagnosed in our Centre. Unfit AML pts (18/55) were selected based on SIE, SIES and GITMO consensus criteria and treated with HMAs. Pts demographics and baseline clinical characteristics were: 9M/9F; *de novo* 9/18 and secondary 9/18 pts; median age 73.5y (range61-86); Performance Score (ECOG) was ≥2 in 14/18 (78%) pts; HCT-CI score was ≥3 in 6/18 (33%) pts. In this subset 13/18 (72%) received decitabine (20 mg /m^2 daily for five days every four weeks) and 5/18(28%) azacitidine (75 mg/m^2 daily for seven days every four weeks. All pts received antibiotic and antimycotic prophilaxis as international guidelines. *Results:* Median HMAs cycles was 12 (range 1-22); OS was 10 months (range 1-26). Up to now 13/18(72%) pts are alive, with a median follow up of 10 months (range 2-26); 11/18 pts were evaluable for response: ORR was 61%: PR in 8/11(73%) and CR in 3/11(27%), stable disease was in 7/18 pts (39%). The total infectious diseases (≥3 grade WHO) occurred in 7/18 pts (39%) consisting in pneumonia (4; 1 fatal) bowel inflammation (2) and necrotizing fasciitis (1 fatal). Two pts died for extraematological causes. *Comments:* SIE SIES GITMO consensus permit to select unfit AML pts who could benefit by HMAs. Encouraging results in this subset confirm the use of HMAs agents in real life. We underline the need to administer antibiotic an antimycotic prophylaxis to prevent serious infections.

P066

SEQUENTIAL THERAPY WITH DASATINIB AND FLAD CHEMOTHERAPY FOLLOWED BY ALLOGENEIC STEM CELL TRANSPLANT IS AN EFFECTIVE AND FEASIBLE STRATEGY FOR PH+ ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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The prognosis of Philadelphia positive (Ph+) acute lymphoblastic leukemia (ALL) patients has improved since the introduction of tyrosine kinase inhibitors (TKI). Following single agent TKIs treatment,all patients achieve complete hematologic remission (CR), but virtually all will eventually relapse without further treatment. On the other hand, the concomitant combination of TKIs to conventional chemotherapy regimens greatly increasescure rate, but at the price of non negligible toxicities. We present here preliminary results of a sequential therapeutic strategy starting with TKI as single agent induction until CR is achieved, followed by consolidation chemotherapy plus TKI and allogeneic transplant (HSCT) if feasible. Dasatinib was given at the dosage of 140 mg /die.Once CR was achieved, FLAD chemotherapy consolidation was started, consisting of a three-days administration of Fludarabine 30 mg/sqm followed by Ara-C 2000 mg/sqm and DaunoXome 100 mg/sqm. Dasatinib and G-CSF were added from day 4. FLAD regimen was delivered for up to 2 cycles. Minimal residual disease (MRD) was evaluated in all patients by multicolor flow cytometry (MFC) and RQ-PCR for BCR/Abl. First MRD assessment was scheduled on day +33. HSCT was scheduled for all eligible patients. From 2008 to 2016 8 Ph+ ALL at diagnosis (median age 52 years) have been treated. Themedian follow-up was 27 months. All patients received at least 45 days of Dasatinib as single agent and achieved CR with complete hematological recovery. In all patients but one BCR/Abl was still positive on day 33. Two patients were MFC MRD positive on day 33, whereas five patients achieved MFC MRD negativity. After the first FLAD course all patients achieved MFC MRDnegativity, with four patients achieving also negativity forBCR/Abl transcript. FLAD was very well tolerated, with a median ANC and platelet recovery of 7.5 and 4 days, respectively. No patient experienced relapse so far. Six patients are alive and in MRD negative CR at the time of analysis. Two patients have died due to non-relapse mortality after SCT. Administering FLAD in patients who had already achieved complete hematological response with Dasatinib+steroids allowed us to reduce the period of neutropenia and thrombocytopenia. Most patients were able to proceed to ASCT in a molecular CR.

P067

A COMPREHENSIVE ANALYSIS OF PATIENT- AND THERAPY-RELATED FACTORS AFFECTING THE TOXICITY OF PEGYLATED-ASPARAGINASE FOR THE TREATMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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The use of intensified regimens for adult acute lymphoblastic leukemia (ALL) treatment has led to a wide use of pegylated L-Asparaginase (PEG-ASP) in adults. Patient-related features as high BMI or hepatic steatosis have been identified as risk factors for PEG-ASP related hepato-toxicity. Few data are available on the additive toxic effect of concomitant treatments. We retrospectively analyzed the incidence of PEG-ASP related adverse events in a cohort of adult ALL patients treated in our institution in order to identify patient and therapy-related risk factors contributing to PEG-ASP toxicity. Since 2013 21 adult patients received PEG-ASP. Median age was 44; 12 patients were treated in frontline setting, whereas 9 patients had relapsed/refractory ALL.

Each course including PEG-ASP was considered an independent event, accounting 41 episodes. No grade III/IV pancreatic, thrombotic or hemorrhagic events were recorded. Five patients experienced grade III hepato-toxicity and 3 patients grade IV. All 3 patients experienced severe weight gain and painful epathomegaly (a clinical picture resembling sinusoidal occlusive disease) and ultrasonography showed acute liver steatosis. In univariate analysis grade III/IV hepato-toxicity was significantly higher when at least 2 mg/sqm cumulative dose of vincristine (p 0.044, HR 4.75) or at least 16 mg/sqm cumulative dose of idarubicine (p 0.046, HR 1.45) were administered. Steroids determined a borderline increase in toxicity risk. No increase in toxicity was observed with any dosing of daunorubicin, cyclophosphamide, cytarabine, methotrexate and 6-mercaptopurine. Among antibiotics, vancomycin administration seemed to increase the incidence of grade III/IV hepato-toxicy (p 0.02, HR 1.863). Age >45, active disease and BMI >25 were not related with an increased incidence of grade III/IV hepato-toxicity. Notably, none of the 7 patients undergoing full pediatric induction, who received the highest doses of PEG-ASP, experienced grade III/IV hepato-toxicity. A multivariate logistic regression analysis showed that administration of idarubicin, vincristine or vancomycin were independent predictors of grade III/IV hepatotoxicity (p 0.004, 0.027 and 0.042, respectively). Our data show that the toxicity profile of PEG-ASP in adult patients is overall manageable. However, concomitant drugs and timing of administration play a crucial role contributing to severe PEG-ASP hepato-toxicity.

P068

EXPRESSION PROFILE OF BONE MARROW MESENCHYMAL STROMAL CELLS ISOLATED FROM PATIENTS WITH THERAPY-RELATED MYELOID NEOPLASMS

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The damage of the bone marrow (BM) microenvironment, in addition to neoplastic transformation of hematopoietic progenitors, contributes to the development to myeloid neoplasm (MN). Functional and morphological abnormalities of BM mesenchymal stromal cells (MSC) have been described in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). MDS- and AML-MSC display impaired expression profile of several genes involved in hematopoietic support, differentiation, senescence, in the PI3K-AKT and WNT pathways, compared to normal controls. Little is still known on the expression of these genes in MSC from therapy-related MN (t-MN). We studied the expression profile of several genes involved in osteogenic differentiation (Osterix and RUNX2), in the interaction with hematopoietic stem cells (Kit-ligand, Jagged1, FOXC1 and CXCL12), belonging to the WNT pathway (SOX9, WISP1, IGF1, EGR1, CCND1), the epigenetic regulation of transcription EZH2 and the oncosuppressor protein TP53 in MSC isolated from 14 t-MN patients and 7 healthy donors. BM-MSC at 2nd passage were used for all experiments. mRNA levels of target genes were analyzed using a semi-quantitative real time. The nonparametric Mann–Whitney U test was used for statistical analysis. t-MN-MSC displayed impaired expression of several genes implicated in osteogenic differentiation. mRNA levels of Osterix (a transcription factor critically involved in the early osteogenic differentiation process) and RUNX2 (regulator of osteogenic differentiation) were detectable in MSC of donors, but not in t-MN MSC. The mRNA expression profile of Jagged1 was significantly upregulated in t-MN-MSC compared to controls (p=0.012). On the contrary no differences were observed in the expression profile of other genes involved in the interaction with hematopoietic stem cells. Concerning WNT target genes, known regulators of multiple MSC properties, no differences were observed in t-MN-MSC compared to controls, while a trend towards upregulation of SOX9 mRNA was observed in t-MN-MSC. EZH2 and TP53 mRNA (p=0.0033, and p=0.0611, respectively) were downregulated in t-MN-MSC compared to controls. There were no differences in the expression profiles when stratifying t-MN according to blast counts. Our preliminary data show significant deregulation of multiple genes implicated in mesenchymal stem cell function in t-MN, which may contribute to MSC dysfunction and may affect their ability to support normal hematopoiesis, participating to t-MN development.

P069

PALB2 ALTERATIONS IN AML PATIENTS CO-OCCUR WITH DNA DAMAGE REPAIR PATHWAY ALTERATIONS AND ASSOCIATE WITH POOR PROGNOSIS

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Partner and localizer of BRCA2 (PALB2) plays a key role in the DNA damage repair (DDR) and its alterations were described in hereditary breast cancer and Fanconi Anemia (FA). Little is known in acute myeloid leukemia (AML). Aim of the study is to define the frequency and interplay of PALB2 alterations with patterns of somatic mutations and copy number alterations (CNAs) in AML. We genotyped 233 AML samples by Single Nucleotide Polymorphism array (Affymetrix) and we performed Exome Sequencing (WES, Illumina) of 56 cases to detect single nucleotide variants and small indels (MuTect and Varscan). Differences in survival were assessed by statistical analysis. We detected PALB2 loss in 12/233 patients (5%), with a minimal common region of 6.6 Kb including exon 12, which encodes for the interaction domain with RAD51, BRCA2 and POLH. PALB2 deletion were correlated with CN loss of CREBBP, PLK1 and FANCA on chromosome (chr) 16 (p≤.005), deletions of TP53, NF1 and BRCA1 on chr 17 (p≤.004) and 5q deletions (p≤.001). PALB2-loss patients were enriched for alterations in genes involved in the protein kinase pathway (p=5 10-4), JUN kinase activity (p=.0006) and in genes of the chromosome breakage pathway (p=.001; TP53, BRCA1 and BRCA2). To identify mutations that co-operates with CNAs, we integrated WES data of 56 AML patients. No mutations in PALB2 were detected. However, we identified mutations in other DDR genes, including FANCE, BRCA2, TP53 and BRCA1. Of note, alterations in the DDR genes co-occurred with both mutations and CN loss of TP53, leading to homozygous loss of function of TP53. In addition, KRAS, IDH1, TET2 and BCOR mutations were mutually exclusive with PALB2 loss. Patients harboring PALB2 loss had a poorer prognosis compared complex karyotype cases (p=.021, Breslow test). In particular, double BRCA1 and PALB2 loss, single BRCA1 or PALB2 hits and lack of alterations in BRCA1 and PALB2 defined three different classes of risks in our AML cohort (p<0.001). We here define a new subset of AML patients, characterized by the synergistic loss of DDR genes. Our data suggest that a signature of genes involved in the DDR and cell cycle regulation may synergize and led to the uncontrolled proliferation, independently of DNA damage accumulation. These results will help improve patient stratification and define synthetic lethal strategies for this aggressive leukemia type. Supported by: ELN, AIL, AIRC, progetto Regione-Università 2010-12 L. Bolondi, NGS-PTL, HARMONY.

P070

ACUTE MYELOID LEUKEMIA WITH MUTATED NPM1 PRESENTING WITH LIFE-THREATENING ARTERIAL OR VENOUS THROMBOEMBOLISM: A REPORT OF 8 CASES

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Background: Large vessel thrombosis is a very rare clinical presentation of acute myeloid leukemia (AML), generally associated with acute promyelocytic leukemia (APL). AML expressing mutated NPM1 gene and cytoplasmic nucleophosmin (NPMc+ AML) is a new entity of WHO classification that shows distinctive biological and clinical features. NPMc+ AML usually presents with hyperleukocytosis and abnormalities in megakaryocytopoiesis. Report Here we report 8 cases of NPM1 mutated AML presenting with life-threatening thromboembolic (either arterial or venous) events (TE). 6 patients out of 8 experienced multiple and/or recurring thrombotic events. All patients (except case nr. 6) lacked any risk factors or personal history of thrombosis. Hyperleukocytosis was a common feature. Platelet count in all of the cases was mildly to moderately decreased. TE (acute myocardial infarction, acute ischemia of right lower limb and venous thrombosis respectively)

was diagnosed before AML in 3 patients (case nr. 1, 2 and 8), while for the remaining ones was concomitant with the diagnosis of leukemia. One patient (case 4) could not be treated for severe concomitant renal failure and died few days after the diagnosis; 7 patients received standard induction therapy despite their critical conditions and achieved complete haematological remission (CR). None of the patients experienced TE after the treatment. *Conclusions:* Large vessel thrombosis is a very rare event at AML onset. TE resolution and CR achievement after chemotherapy highlights the life-saving role of prompt treatment even under adverse circumstances. TE pathophysiology in haematological malignancies is complex and multifactorial for clotting formation and regulation, entailing tumor cell-derived procoagulant, fibrinolytic/proteolytic factors and inflammatory cytokines. Other important promoters are infections and hyperleukocytosis. Our single-center experience suggests that TE occurrence at leukemia onset could be preferential for NPMc+ AML subtype likely due to abnormal platelet production/function and other intrinsically biological aspects still to characterized.

Table 1. Characteristics of patients with NPM1-mutated AML and thrombosis.

Case report n°	Age	Sex (M/F)	FAB subtype	WBC/mm c	PLT/mmc	Type of thrombosis	Site of thrombosis
1	41	F	M1	35220	64000	arterial	Anterior interventricular branch of left coronary artery
2	56	M	M4	77000	40000	arterial	external iliac and femoral (right limb)
3	63	M	M2	105100	38000	venous	great saphenous veins (bilateral)
4	73	F	M4	260000	NA	venous	iliac and femoral
5	67	M	M1	142600	96000	arterial	Left hemispherical ischemic stroke
6	59	F	M4	124100	25000	arterial	Anterior acute myocardial infarction, left hemispherical ischemic stroke
7	43	F	NA	83370	112000	venous	Left subclavian vein, superficial thrombophlebitis
8	59	F	M4	7050	39000	venous	Left great saphenous vein, diffuse involvement of right leg deep venous circle

P071

PROGNOSTIC SIGNIFICANCE OF ALTERATIONS IN PATHWAYS REGULATING AUTOPHAGY IN ACUTE MYELOID LEUKEMIA

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Introduction: To date, cancer research is debating if autophagy may lead to therapy resistance or favor apoptosis. Autophagy pathways are involved pro-apoptotic mechanism, but they can also improve stresses survival eliminating damaged mitochondria and proteins. Levels and activity of pro-apoptotic and anti-apoptotic proteins (eg. bcl-2 and p53), high levels of cAMP, and a complex made by pink/park could play as fulcrum on this lever. Our study aims to define the role of autophagy in AML. *Methods:* We analyzed bone marrow samples from 148 non M3 AML patients at diagnosis with Affymetrix SNP array 6.0 or Cytoscan HD. All the patients weres treated with intensive induction hemotherapy regimens. We screened all patients for TP53, FLT3, NMP1 mutations. Survival data were collected prospectively, with a median follow-up of 18 months. *Results:* Autophagy alteration (figure, gene group 1: ULK1 CHR11; ULK1 CHR17; BECN1; ATG14; AMBRA1; UVRAG; ATG9A; ATG9B; PIK3C3; PIK3R4) was related to lower Complete Remission rate after induction chemotherapy (CR%) in univariate (p<.001) and multivariable logistic regression model with age, karyotype, secondary AML, TP53 mutation (p=.014); autophagy alteration shown to confer worst OS (p<.001) and was significantly associated with complex karyotype and TP53 mutation (p<.001). We detected significant differences in term of survival independently both in gain and loss in group 1 genes (p<.001). Alterations in genes in cAMP pathway (group 2: SESN1; PRKAA1 CHR 3; PRKAB1: PRKAA1 CHR 1: PRKAG1 CHR11; PRKAG1 CHR 7; PRKAG3; PRKAB1) and in genes that could be related to a switch from a physiological role of autophagy to a resiliency mechanism (group 3: CCND1; BCL2; PINK1; PARK2; TP53; MDM1; MDM4) showed to confer worst OS (p<.001 in both groups); Alteration in group 2 and group 3 were related to lower CR% (p<.001 in both groups). Whole Exome Sequencing on 56 patients in our set did not found any significant mutation in genes we analyzed with the exception of TP53. *Conclusions:* Our work investigates for the first time the role of autophagy in AML patients with a genomic aproach. Alterations in autophagy key regulator genes are associated with poor prognosis and therapy resistance. A loss in autophagy could block apoptosis; a gain could confer cell resiliency. *Acknowledgements:* ELN, AIL, AIRC, PRIN, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project, HARMONY project.

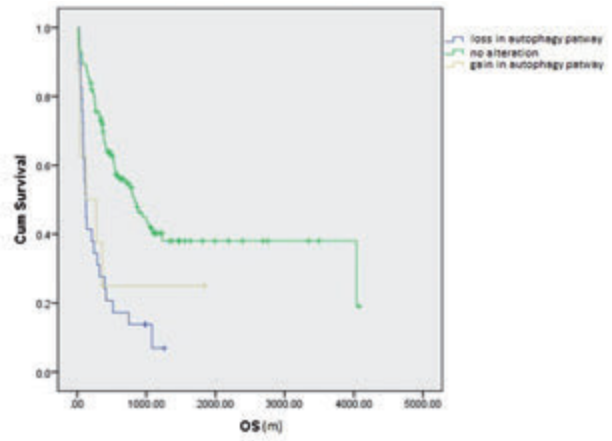

Figure 1.

P072

DECITABINA NEL PAZIENTE ANZIANO CON LEUCEMIA ACUTA MIELOIDE: INTERIM ANALYSIS DELLO STUDIO OSSERVAZIONALE ITALIANO MULTICENTRICO DEA65

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Older patients (pts) with Acute Myeloid Leukemia (AML) have a worse prognosis and limited treatment options. Hypomethylating agent decitabine was recently approved by FDA and EMEA as first line treatment in AML pts older than 65 yrs and unfit to standard chemotherapy. In July 2016 we started a retrospective and prospective multicentric observational study to investigate in real life efficacy and tolerability of decitabine at the approved schedule of 20mg/m^2 daily for 5 days every 4 weeks (DEA65 study). Ninety-five pts, with a median age of 75 yrs (range 65-90) were enrolled. At diagnosis and during follow-up, cytogenetic and molecular assessment was performed by each center according to local guidelines for AML management in elderly pts. Fifty patients (52,6%) had a secondary AML and 19/50 were progressed MDS previously treated with 5-azacitidine. Median WBCs count was 3240/µL (range 600-131500) with 25/95 (26,3%) pts with WBCs >10000/µL. According to prognostication, 45,5% of pts had a high risk, 28,5% an intermediate risk, 6% a low risk AML and in 19/95 (20%) pts risk was unknown. Median OS was 8 months (range 1-28) with 57/95 deaths (60%) and a median of 6 cycles (range 1 to 20) of decitabine. Overall response rate was 50,5% (48/95 pts), of which 10/95 (10,5%) CR or Cri; 23/95 (26,3%) PR and 15/95 (15,7%) improvement of transfusion needs. According to response, median OS was 10 months (range 4-20) and 4 months (range 1-16) in responder *vs* not responder pts. At

present time 28/38 alive pts are still on treatment with decitabine. Regarding toxicity, 38% pts manifested a grade ≥3 non-hematologic AEs (more frequently pneumonia and fever) although severe comorbidities (cardiovascular and metabolic) pre-existed in 21/37 (56,7%) and 20/95 pts (21%) died due to serious AEs. This interim analysis of the use of decitabine in real life showed a superimposable OS to controlled international clinical trials (Kantarjian, JCO 2012; Cashen, JCO 2010). Considering setting of pts safety profile was acceptable. Despite a similar OS, the comparison between our data and international studies showed in our cohort, a poorer rate of CR+CRi with a negative impact of secondary AML, previous 5-azacitidine therapy, WBC>10000/µL as well as high cytogenetic risk. This apparent contradiction supports the idea that in elderly pts recovery of peripheral blood cells counts (PR+hematological improvement) is probably the most important factor influencing OS (Ferrara, Hemat 2016).

P073

SURVIVAL ANALYSIS OF PATIENTS CARRYING DIFFERENT FLT3 MUTATIONS (INTERNAL TANDEM DUPLICATION (ITD) AND TYROSINE KINASE DOMAIN (TKD) MUTATIONS) IN 459 CONSECUTIVE NON M3 NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA (AML)

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Background: A subset of acute myeloid leukemia (AML) is characterized by internal tandem duplication (ITD) of FLT3 gene. The impact on prognosis of FLT3-ITD is well known, but different lenght of ITD were never correlate to prognosis. Our study aims to define effect on survival of ITD lenght. *Methods:* In a cohort of 459 consecutive AML patients, we analyzed FLT3-ITD at the diagnosis with qualitative PCR, followed by a mutational screening with Denaturing High Pressure Liquid Chromatography and Sanger sequencing in positive patients, in order to characterize the kind and the length of the ITD. Survival analysis was performed with Kaplan-Meier method using Mantel-Cox test. *Results:* At diagnosis, 59 out of 459 patients (12.0%) harbored ITD of FLT3 gene; 4/59 of FLT3-ITD patients had concomitant thyrosine kinase domaim mutation. Median age of the 59 patients that harbored FLT3-ITD mutations was 57 years (range 21-83).

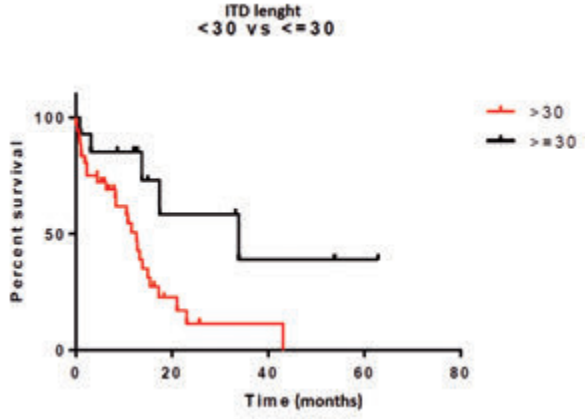

Figure 1.

At diagnosis, median white blood cells count (WBC) was 44000/mmc (range 8900–234000); karyotype was evaluable in 51/59 patients. Eight out of 51 patients had complex or monosomic karyotype, 29/51 patients had normal karyotype and 14/51 patients had other karyotype alterations. All those patients except 5 had a newly onset leukemia, in 1/59 patients, leukemia was secondary to chemotherapy, in 1/59 patients to chronic myelomonocitic leukemia, in 3/59 patients was secondary to myelodysplasia. Within FLT3-ITD patients, median overall survival(OS) was 13.27 months. In this set, we considered the length of the duplication/insertion: fifteen out of 59 patients harbored an ITD involving a low number of nucleotides (ITD minor of 30 nucleotides) and they also showed a better outcome in term of overall survival compared with the 40/50 patients harboring ITD involving high number of nucleotides (ITD equal or major to 30 nucleotides) (median OS was 33.8 and 12.6 months in the 2 groups, respectively, p=0.009). Patients with shorter ITD mutations showed a lower median WBC if compared with patients with longer ITD, 29850/mmc *vs* 46100/mmc respectively. *Conclusions:* In AML, FLT3-ITD mutation defines a poor prognosis. No study proved the importance of ITD length or position. In our set, we showed that patients with worse prognosis are identified by ITD major or equal to 30 nucleotides. *Acknowledgements:* ELN, AIL, AIRC, PRIN, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project, HARMONY project.

P074

SEPARASE OVEREXPRESSION DEFINES A NEW SUBSET OF ACUTE MYELOID LEUKEMIA PATIENTS

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The endopeptidase Separase, encoded by the ESPL1 gene, plays a key role in faithful segregation of sister chromatids by cleaving the cohesin complex at the metaphase to anaphase transition. Its overexpression associates with aneuploidy and bad prognosis in solid tumors. Little is known in Acute Myeloid Leukemia (AML). We profiled the genomic landscape of 405 and 78 AML cases by SNP array (SNP 6.0 and Cytoscan HD, Affymetrix) and whole exome sequencing (100 bp, paired-end, Illumina), respectively. Bone marrow (BM) blasts from 61 patients were analyzed by gene expression profiling (HTA 2.0, Affymetrix). Separase expression was determined by Immunohistochemistry (1:600 antibody dilution Abnova, clone 6H6) in 44 AML and 4 control BM specimens. One patient exhibited a nonsynonimous mutation in ESPL1 (1.3%), predicted to alter the protein function. ESPL1 copy number gain was observed in 5/405 cases (1.2%): 2 hyperdiploid AML, one trisomy 12 and 2 cases with a short gain at 12q. Notably, protein level detection in one of the 12q-gain cases confirmed Separase overexpression. We performed Immunohistochemistry on additional 43 AML. Separase was overexpressed in 29/44 AML (66%, Separase-high) comparable to control BM in the remaining 15 samples (Separase-low). Separase overexpression correlated with increased patients' age, 17-fold upregulation of CD34 (p=.004) and a trend towards reduced overall survival (6-years follow-up). Separase overexpression co-occurred with NPM1 and FLT3 lesions and mutations in genes involved in protein post-translational modification and ubiquitination. Separase-low cases were enriched for mutations in RAS signaling pathway (NRAS, KRAS,NF1, RIT1, GRAP2, RALGDS; p=4.5x10-5) and in cell migration-related genes (LIMS2, S1PR1, PPIA, PLXNB1, FAT1). Separase-high cases also showed a defined transcriptomic profile, characterized by reduced expression of HOXA/B family genes, the DNA damage repair gene ATM, the p53 regulator MDM2 and forced expression of the cell cycle markers CDC20, AURKB, NUSAP1 and of MYC, independently of chromosome 8 gain. Our data suggest that genomic lesions targeting ESPL1 are a rare event in AML. Separase overexpression is a common feature and defines a new subset of AML cases with a distinct gene expression profile, which may benefit of innovative targeted therapies including CDC20 and bromodomain inhibitors. Supported by: ELN,AIL,AIRC,progetto Regione-Università 2010-12 (L. Bolondi),FP7 NGS-PTL project,HARMONY.

P075

ALTERATIONS IN PHOSPHATIDYLINOSITOL 3-PHOSPHATASE (PI3P) PATHWAY AND CAMP PATHWAY CONFER POOR PROGNOSIS AND REDUCE OVERALL SURVIVAL (OS) IN A SERIES OF 208 NEWLY DIAGNOSIS ACUTE MYELOID LEUKEMIA (AML) PATIENTS

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Introduction: PI3P regulate cell growth and mediates cell proliferation via PI3K/AKT/mTOR in response to various growth signals. Abnormalities in genes in its pathways are associated to oncogenic activity and poor Overall Survival (OS). AMPK plays a role as a regulator of cellular energy homeostasis. The aim of the this study is to define the role of PI3P pathways and AMPK pathway in AML. *Methods:* In this work we analyzed 208 consecutive newly diagnosed non M3 AML patients, screened for TP53, FLT3, NMP1, IDH1, IDH2, and DNMT3A mutations. Remission status was assessed with bone marrow biopsy. We performed Microarray-based Comparative Genomic Hybridization with Affymetrix SNP array 6.0 or Cytoscan HD in all the patients; we performed Whole Exome Sequencing (WES)in 80/208 patients. Survival data were collected prospectively, with a median follow-up of 18 months. Survival analysis was performed with Kaplan Meyer method using log rank test. Univariate and multivariable regression and Cox Hazard Ratio(HR) model was performed. Correlation between variables was assessed with Fisher's exact test. *Results:* We selected genes in pathways basing on literature and GO data. Alterations in these pathways involved 103/208 patients (48%). We analyzed the gene in two different pathways. PI3K/AKT/mTOR pathway includes the following genes: pik3ca, cdkn1a, akt1, akt3, mtor and pten, pdk1,pik3r1 and irs1. AMPK pathway include: sesn, prkaa1, prkab1, prkag1, prkag3. Alterations in PI3K/AKT/mTOR pathway confer worst OS (p=.035) when compared with unaltered patient, but events in these pathways did not affect therapy response. Alterations in AMPK pathway confer worst OS (p<.001); Alteration of regulators in cAMP were related to lower complete remission rate after induction in univariate (p<.001) and multivariable analysis with age, karyotype, secondary AML, TP53 mutation (p=0.009). AMPc pathway alteration was significantly associated with complex karyotype and TP53 mutation (p<.001). WES in a sub-cohort of patients did not found any significant mutation in genes we analyzed, according to literature. *Conclusions:* Our work investigates the role of PI3P and cAMP pathways in AML. We showed that alterations in these pathways are associated with poor prognosis. Significantly, alterations in cAMP pathways were associated with therapy resistance. *Acknowledgements:* ELN, AIL, AIRC, PRIN, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project, HARMONY project.

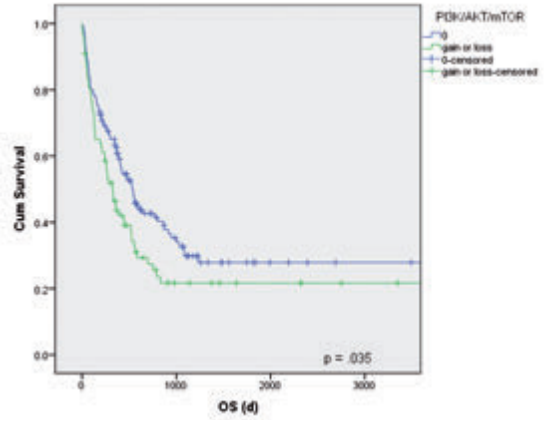

Figure 1.

P076

EFFICACY AND FEASIBILITY OF FULL PEDIATRIC INDUCTION IN PH-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA ADULT PATIENTS

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IRCCS AOU San Martino-IST

Most recent studies suggest that young adults with acute lymphoblastic leukemia (ALL) benefit of pediatric rather than adult chemotherapy regimens. Pediatric schedules provide an increased drug intensity at several treatment steps, and a tighter evaluation of minimal residual disease (MRD) for MRD-driven intensification. Our aim was to evaluate efficacy and feasibility of a full pediatric induction in a cohort of adult patients with Ph-neg ALL. Since May 2013, 9 consecutive patients (median age 25, range 19 to 53) with Ph-neg ALL (5 B-ALL, 4 T-ALL/T lymphoblastic lymphoma) received first line therapy according to AIEOP-BFM-ALL 2009 full pediatric protocol. Dose intensification and bone marrow transplantation (BMT) were scheduled according to baseline and MRD-driven risk assessment. MRD time points were day 15 (MFC only), day 33 and day 74 (PCR for JH rearrangement). Fresh frozen plasma was generally not administered in order to avoid asparagine repletion. At day 15 MFC MRD was less than <0,025% (undetectable) in 2 patients, less than 10% in 4, more than 10% in 3; at day 33 and 74, MFC MRD was undetectable in 6/7 and 5/5 patients respectively. At day 33, 3/6 patients achieved complete molecular response (JH <10-5), 2 had partial molecular response (JH <10-3), 1 patient still had higher MRD levels. At day 74 all surviving patients achieved JH MRD negativity, that was maintained during follow up (Figure 1). One patient died during induction due to severe infection by multidrug resistant P. aeruginosa; at that time he was in complete molecular response with normal blood counts. Two very high risk patients at diagnosis underwent BMT in complete molecular response. After a median follow up of 24 months, overall survival was 85%. No relapses occurred. In general, therapy was well tolerated without excess of toxicity. Patients receiving higher dose of pegilated asparaginase (pegASNase) experienced more extra-hematological toxicities, however, no major pancreatic or thrombotic complications requiring treatment suspension were observed. In our experience the application of a full pediatric induction regimen in a small cohort of adult ALL patients provided good results at the price of only mild toxicities. Specifically we didn't observe major toxicities during the intensified pegASNase administration and all patients received the planned pegASNase doses. MRD clearance rate was promising, as all patient at the end of induction achieved complete JH MRD negativity.

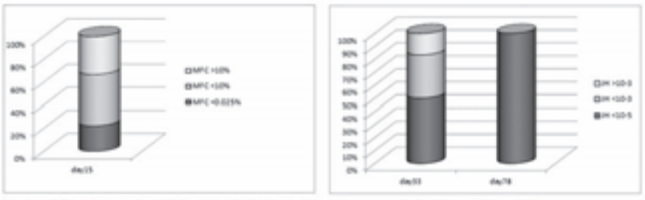

Fig.1 MRD evalutated in MFC and in RT-PCR for JH rearrangement at different timepoints.

Figure 1.

P077

MOLECULAR MONITORING OF NPM-1 MUTATION IN NPM1-MUTATED ACUTE MYELOID LEUKEMIA IDENTIFIES A HIGH PROPORTION OF LATE RELAPSES ALLOWING A SAFE AND EFFECTIVE USE OF ALLOGENEIC TRANSPLANT. AN ANALYSIS OF 63 PATIENTS AT A SINGLE INSTITUTION

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Monitoring of minimal residual disease (MRD) is becoming an important tool to predict early relapse in patients (pts) with acute myeloid leukemia (AML), even in pts with good prognosis like NPM1-mutated AML. Some studies have shown that NPM1 mutation (NPMm) is a stable molecular marker for MRD. We evaluated the outcome of 63 pts with NPM-1 mutated (NPMm) AML [M/F:35/28; median age 54y (27-74)] identified among 185 *de novo* AML diagnosed at our Institution from Jun 2010 to Nov 2016. Quantitative NPMm analysis, performed by RT-PCR (Gorello 2006), was studied in blood and bone marrow at diagnosis, during and at the end of treatment, and during the follow-up. The cut-off for diagnosing molecular relapse (mR) was NPM>200 copies/Abl (Kronke, 2011). NPMm pts received HD-ARAC-based program according to NILG AML00 protocol (Bassan,2003). Karyotype was normal in 55 pts (87.3%). FLT3-ITD was detected in 24 pts (38%). Complete remission (CR) was achieved in 62 pts (98.4%), one pt died during aplasia (1,6%). Twenty of 62 pts (31.7%) relapsed after a median of 16.6 mo (1-61.5); 11 were haematological relapses (hR) and 9 mR. Seven hR occurred during treatment (median 3,6 mo),defined as early relapse (ER). Four hR and all 9 mR occurred off treatment (Tx) and were

defined late relapses (LR) (median 20.9 mo). Age, NPMm burden at baseline, FLT3 positivity, chromosomal aberration, and time to NPM negativization did not influence relapse risk. However 6/7 ER pts had FLT mutation. While MDR monitoring was not useful to identify ER it allowed to identify 9/13 pts with LR. Relapse could be treated in 18/20 pts. Of 9 mR, 6 were treated with not intensive (ni-T) (2 ATRA, 4 D-act) for a median of 3 cycles and 3 with intensive T (i-T). No pts progressed to hR and all 8 eligible pts underwent ASCT. Of 9 hR pts, 7 received i-T and 2 ni-T with D-act. Two CR were obtained (1 with niT). Five hR pts could be allotransplanted, only 2 in CR. The median follow-up was 33 mo (range 1-86). Median overall survival was 61.5 mo. At 5-y, DFS was 62+/-11% and OS 71+/-8%. Median survival was 7.1 mo in ER pts and 52.3 mo in LR pts. The study confirms the overall good prognosis of NPM AML, except for some pts with coexistent FLT3-ITD mutation who and a dismal prognosis. The study further shows that NPM monitoring allowed to timely identify relapse at the stage of mR in 69% of relapsing pts off treatment and to bring them to ASCT while still in hematological CR, even by using less toxic niT.

Chronic Myeloid Leukemia 1

P078

ESTABLISHING A NATIONAL NETWORK OF LABORATORIES PERFORMING BCR-ABL KINASE DOMAIN MUTATION SCREENING BY NEXT GENERATION AMPLICON DEEP SEQUENCING: RESULTS OF THE 'NEXT-IN-CML' STUDY

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A multicenter, multilaboratory prospective study ('NEXT-IN-CML') has been conducted to assess the feasibility, cost, turnaround times and clinical utility of a next generation amplicon deep sequencing (Deep Seq) strategy for routine BCR-ABL mutation screening of chronic myeloid leukemia (CML) patients (pts) on tyrosine kinase inhibitor (TKI) therapy. The 1st phase of the study was aimed to i) create a network of 4 reference labs sharing a common protocol, a joint database for clinical and mutational data storage and a common pipeline of data analysis, interpretation and reporting and ii) verify accuracy and interlaboratory reproducibility of Deep Seq results. The 2nd phase of the study, involving 39 Hematology Units, was meant to prospectively assess the frequency and clinical significance of low burden mutations in CML pts with Failure (F) or Warning (W) response to any TKI. In the 1st phase, centrally prepared identical batches of 32 blinded specimens (clinical samples+cell line dilutions) were distributed and analyzed in parallel by each lab. 51/52 expected mutations were consistently detected by all labs and quantitation of mutation load was highly reproducible across a wide range of frequencies (2%-100%). 3/4 labs failed to detect the 1% T315I dilution and 2/19 low burden mutations <3% present in clinical specimens were missed by 2 labs, suggesting that 3% should be taken as a threshold below which mutation detection is not always reliable and reproducible. In the 2nd phase, 189 consecutive CML pts (121 F and 68 W) were prospectively studied in parallel by Sanger Seq and Deep Seq. Pts positive for mutations were 34/189 (18%) by Sanger Seq and 88/189 (47%) by Deep Seq. Low burden mutations were found in 54 pts negative by Sanger seq and 23 pts with other mutations detectable by Sanger seq. 5 pts had a low burden T315I; 41 had other known TKI-resistant mutations; 31 had mutations with unknown sensitivity profile. Detailed analysis/correlations will be presented. *Conclusions:* 1) Results of the first prospective study evaluating the routine diagnostic use of Deep Seq of BCR-ABL show that this technology can successfully be implemented in national lab networks and is feasible, robust and reproducible; 2) in pts who need to be switched to another TKI, BCR-ABL mutation screening by Deep Seq may detect mutations relevant to TKI choice. However, the clinical significance of low burden mutations with unknown resistance profile remains to be established.

P079

LONG-TERM SAFETY IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH IMATINIB. SINGLE INSTITUTION ANALYSIS AFTER A MEDIAN FOLLOW-UP OF 9 YEARS

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Background: Long-term morbidity and mortality analysis in chronic phase chronic myeloid leukemia (CP-CML) patients treated with imatinib has been reported only in sponsored clinical trials. Only the ILTE study reported an independent analysis after a median follow-up of 5.8 years, considering only patients in complete cytogenetic response. *Aim:* We investigate the incidence of adverse events and CML-related and -unrelated deaths in a large series of CP-CML patients treated with imatinib at a single institution. *Patients and Methods:* Overall, 320 patients were analyzed after a median follow-up of 9 years. The incidence of serious and non-serious adverse events was retrospectively recorded through patients' charts, including deaths and second cancers. Side effects were graded according to the CTC scale. *Results:* We recorded 50 CML-unrelated deaths (14.7%), the majority of which were related to cardiovascular diseases (17 patients) and senectus (13 patients): 9 patients died of a second neoplasia, 4 due to cerebral hemorrhage, 4 of infection, 2 due to a chronic renal disease and 1 patient of intestinal occlusion. In all cases, a correlation with the underlying disease was excluded. Twenty-four patients were recorded as CML-related death (7%). Of 255 patients who remained on treatment, 33 (13%) experienced a cardiovascular side effect: a thrombotic event (cardiac, cerebral or PAOD) in 12 of the 33 patients, an arrhythmia in 9 and other cardiovascular complications (pericarditis, heart failure, etc) in 12. All patients continued treatment after the event and only 13 temporarily discontinued imatinib; 5 patients continued at a reduced dose. The most common non-serious adverse events recorded were grade 1-2 muscle cramps (8%), gastro-intestinal complications (6%), periorbital oedema (3.5%), arthralgia/muscle pain (3%), congiuntival hemorrhages (2.7%), diabetes (1.1%), chronic renal failure (0.7%), COPD (0.7%), gynecomastia (0.7%). The occurrence of a second neoplasia has been estimated at 2.7%, the most commonly involved sites being the genitourinary tract and the breast. *Conclusions:* The long-term follow-up (median 9 years) of CML patients treated with imatinib indicates that CML-related deaths are uncommon and that the incidence of non-serious adverse events is 30%, remaining constant over time. No new safety signals were recorded. Our findings are in line with those reported in the long-term follow-up of the IRIS trial and highlight that a long-term observation is needed in CML patients treated with imatinib.

P080

ESTIMATED GLOMERULAR FILTRATION RATE IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED FRONTLINE WITH AVAILABLE TKIS AND CORRELATION WITH CARDIOVASCULAR EVENTS

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The long-term effect of available TKIs on renal function has not been described in detail. We investigated the median eGFR changes in CML patients treated with frontline TKIs. A large cohort of 397 patients was retrospectively analyzed at a single institution. The eGFR was calculated according to the Chronic Kidney Disease Epidemiology Collaboration equation for all patients at baseline and then at 6, 12 months, and at the last follow-up. Considering eGFR changes during the first year of treatment and excluding other possible cardiovascular risk factors, we considered the percentage of cardiovascular events in patients with modifications of this parameter. We evaluated 320 patients treated with frontline imatinib, 24 with dasatinib and 53 with nilotinib. The median period of observation is 9 years for imatinib and 2.5 years for second generation TKIs. Imatinib induced a decrease in median eGFR from 81ml/min at baseline, to 78 ml/min at 6 months, 77 ml/min at 12 months and 71 ml/min at the last follow-up (p=0.01). Forty-two patients treated with imatinib had a cardiovascular event: in these patients, the median eGFR decreased from 75 ml/min at baseline to 67 ml/min at the last follow-up (p=0.04). Fourteen of the 42 patients (median age 57 years) had an ischemic event: eGFR modifications seem to be the only possible association in the absence of other concomitant cardiovascular risk factors. In patients treated with nilotinib, the median eGFR did not decline from baseline, starting from 98 ml/min at baseline and becoming 107 ml/min at 6 months, 94 ml/min at 12 months and 92 ml/min at the last follow-up (p=0.11). Only 1 patient experienced an ischemic event, but the eGFR remained unchanged. In patients treated with dasatinib, the mean eGFR started from 93 ml/min then declined to 83 ml/min at 6 months, to 81 ml/min at 12 months and to 80 ml/min at the last follow-up (p=0.167). Even if a decline was noted, statistical significance was not reached due to the small series of patients analyzed. Three patients experienced a cardiac ischemic event, but in none of the patients the eGFR changed over time, while advanced age and metabolic alterations contributed to the ischemic event. Long-term follow-up has documented that imatinib may induce changes in the eGFR, which may contribute to the onset of ischemic events. Further analyses on large series of patients treated with second generation TKIs are required to evaluate the potential renal toxicity and the consequent increased risk of developing ischemic events.

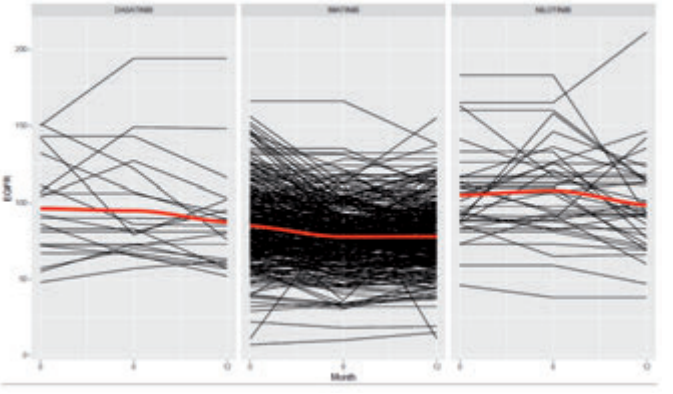

Figure 1. eGFR changes with 3 available TKIs.

P081

TIMING AND DEEPNESS OF RESPONSE TO TKIS AS A MEASURE OF POTENTIAL TREATMENT DISCONTINUATION IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Achievement of a stable and deep molecular response in chronic phase chronic myeloid leukemia patients (CP-CML) has become a prerequisite for a possible treatment discontinuation. A real-life quantification of how many patients outside clinical trials can attempt a treatment discontinuation strategy has never been reported. We estimated in CP-CML patients treated frontline with available TKIs outside clinical trials how many reached a deep molecular response and meet therefore the criteria for a discontinuation. We evaluated 397 CP-CML patients treated with imatinib (320 patients, median follow-up 9 years) or with second generation TKIs (24 patients treated with dasatinib and 53 with nilotinib, median follow-up 2.5 years). RQ-PCR was performed according to the standard international procedures suggested by the European LeukemiaNet. We also evaluated the impact of control gene amplification in the stability of molecular response. Imatinib induced MR4-4.5 in 190 patients (59%) after a median time of 109 months: a stable response was maintained in 133 patients (undetectable in 93). A fluctuation due to amplification of the control gene was observed over time in 49 patients (25.7%). After a median follow-up of 9 years, 41% of imatinib-treated patients could attempt a discontinuation (29% considering undetectable responses). In the cohort of dasatinib-treated patients, 11/24 patients (46%) reached MR4-4.5 after a median time of 23

months: 7 patients maintained the same stable response at the last follow-up (undetectable in 4). Only 2 patients had fluctuations due to control gene amplification in the intermediate test. After a median follow-up of 2.5 years, 29% of dasatinib-treated patients can be defined candidates for discontinuation (17% considering undetectable responses). In the cohort of nilotinib-treated patients, 26/53 (49%) patients reached MR4-4.5 after a median time of 25 months. Twenty-four patients maintained the same stable response at the last follow-up (undetectable in 12 patients). Two patients had unstable response due to amplification of the control gene. After a median follow-up of 2.5 years, 45% of nilotinib-treated patients could attempt a discontinuation (23% in undetectable responses). Imatinib induced deep molecular responses over a longer median time as compared to second-generation TKIs that increased the percentage of possible candidate to discontinuation in a median shorter time. Fluctuations of molecular level due to amplifications of the control gene may influence the estimated rate of patients that could be defined candidate to discontinuation.

P082

EUTOS LONG-TERM SURVIVAL SCORE (ELTS) BETTER DEFINES THE RISK OF DEATH FOR CHRONIC MYELOID LEUKEMIA PATIENTS TREATED OUTSIDE OF CLINICAL TRIALS

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The Eutos long-term survival score (ELTS) stratifies chronic phase chronic myeloid leukemia (CP-CML) patients according to age, percentage of peripheral blasts, spleen size and platelet count in order to establish the risk of disease related-death. A comparative performance analysis between ELTS and other prognostic scores in real-life cohorts of patients treated outside of clinical trials has so far not been carried out. A cohort of 417 CP-CML patients diagnosed at the Sapienza University was included in this study. We evaluated the ability of ELTS to predict the risk of disease-related death compared to the other specific prognostic scores routinely used (EUTOS, Sokal and Hasford). The ELTS score was applicable to 339 patients treated with imatinib and to 78 patients treated with a second generation TKI. All patients had been also stratified according to the other prognostic scores. After a median follow-up of 119 months (range 2-343), 75 deaths were observed among the 339 patients who received imatinib [25 CML-related (33.3%) and 50 CML-unrelated (66.7%)].

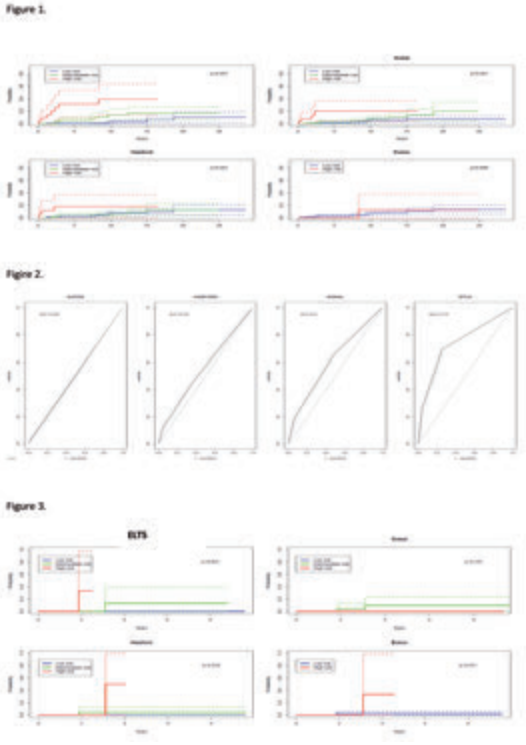

Figure 1.

According to the ELTS score, 241 (71.1%) patients were stratified as low risk, while 77 (22.7%) and 21(6.2%) were categorized as intermediate risk and high risk, respectively. The cumulative 10-year incidence (CI) of CML-related deaths was 2%, 15% and 40%, respectively, for patients belonging to the low, intermediate and high-risk ELTS score ($p<0.001$). Also Sokal score retained its predictive value for the risk of death ($p=0.007$). Indeed, we did not find a statistical significance for the Hasford ($p=0.221$) or Eutos score($p=0.840$) (Figure 1). The receiver operating characteristic (ROC) analysis, showed that ELTS (AUC 0.737) performed better than the Sokal (AUC 0.61), Eutos (AUC 0.505) and Hasford (AUC 0.557) scores in predicting the risk of CML-related death (Figure 2). Among the 78 patients who received second-generation TKIs, we observed only 2 deaths for CML progression and 2 deaths related to other causes after a median follow-up of 39 months (range 4-156). In this subset of patients, according to ELTS, 61 (78.2%) patients were stratified as low risk, 14 (17.9%) as intermediate risk and 3 (3.9%) as high risk. At 2 years, the CI of CML-related deaths was 36% for high risk patients, while the CI was 15% and 0% for patients stratified as intermediate and low risk, respectively ($p<0.001$) (Figure 3). The results show that, outside of clinical trials, the ELTS score stratifies more accurately CML patients at diagnosis compared to the other scores and that its use in the clinical practice should be recommended.

P083

PLEURAL EFFUSION IN DASATINIB-TREATED CML PATIENTS: INCIDENCE AND MANAGEMENT IN A REAL-LIFE ITALIAN MULTICENTER SERIES

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Introduction: Dasatinib (DAS) is a dual BCR-ABL1 and SRC inhibitor approved for the first- and second-line treatment of chronic myeloid leukemia (CML) with a distinct toxicity profile that includes pleural effusion (PE), the latter representing the leading cause of DAS discontinuation. PE may occur through potent inhibition of the PDGFR-, leading to a reduction in interstitial fluid pressure, or inhibition of SRC-family kinases, resulting in vascular permeability changes. However the exact pathogenic mechanism of this adverse event (AE) still remains unknown. *Aim:* To evaluate incidence and management of PE in a real-life DAS-treated CML population. *Methods:* Data were collected in 21 Italian hematological centers. Globally we identified 184 cases of PE in a

series of 790 DAS-treated CML-chronic phase patients (incidence: 23.3%), with a median follow-up of 7.9 years (range 0.1-26.0). Male:female ratio was approximately 2:1 with a median age at diagnosis of 60.7 years. *Results:* DAS starting dose was 100 mg QD in 71.2% of the patients, less than 100 mg QD in 14.7% and more than 100 mg QD in the remaining cases. Median time from DAS start to PE was 16.4 months (range 0.3-109), with 63.1% of cases within the first two years. In 62.5% of the cases PE severity was graded as 2 according to CT-CAE. At the time of PE, 28.2% of patients showed a MMR, and 37% a deep molecular response (DMR); a response less than MMR was reported in the remaining cases. DAS was temporary interrupted in 73.7%, with a dose reduction in 59.8% of the cases. Recurrence was observed in 60.1% of the cases, with a median time from the first episode to the subsequent one of 8.9 months (range 0.5-69.9). Treatment was definitively discontinued in 56.5% of the cases. Interestingly, among patients whose DAS dosage was reduced, 59.1% experienced PE recurrence. *Conclusions:* Comparing our data to that reported in the literature, we identified a MMR and a DMR response rate at the median time of PE superior to that of both first- and second-line DAS-treated CML population (globally 51% at 12 months in the DASISION and 37% at 24 months in the CA180-034 study), also distinguishing between imatinib-intolerant and resistant cases. Furthermore, it has to be highlighted a high PE recurrence rate also after DAS dose reduction. Therefore, considering the latter aspect together with the confirmed DAS efficacy, it could be useful to consider a DAS dosage modulation as soon as a confirmed DMR is achieved in order to prevent this AE.

P084

TYROSINE KINASE INHIBITORS DISCONTINUATION IN CHRONIC MYELOID LEUKEMIA: A RETROSPECTIVE ANALYSIS OF 208 ITALIAN PATIENTS

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Background: Different studies analyzed the outcome of patients (pts) who discontinued tyrosine kinase inhibitors (TKIs) reporting a median treatment-free remission (TFR) rate of 55%. On these bases it is judged safe to discontinue treatment in experimental contexts. *Aims:* To evaluate TFR in the setting of clinical practice. Methods We retrospectively collected and analyzed the outcome of pts treated in 32 Divisions of Hematology in Italy, who discontinued TKIs in CMR. Results We analyzed a total of 292 pts who discontinued TKIs. Median age at stop was 59 yrs (IQR, 47; 70). 160 (55%) were male; 58%, 31% and 11% were low, intermediate and high Sokal score respectively. 161 pts (55%) discontinued in first line; 117 pts (40%) in second, 13 pts (5%) in third, and 1 pt in fourth line. 210 pts (72%) were on treatment with imatinib, 28% with either nilotinib (58), dasatinib (23), bosutinib (1) at the time of discontinuation. Median duration of treatment with the last TKI was 77 mos (IQR 54; 111), median duration of CMR was 46 mos (IQR 31; 74). At 3 mos of last TKI 34% of pts were in MMR, 25% were in PCyR and/or had a transcript < 10%, 40% were in CCyR and/or had a transcript < 1%, and 1% had no response. Responses at discontinuation were: 35% MR4, 31% MR4.5, 18% MR5. 16% were defined as "undetectable". Reasons for discontinuation were: toxicity for 20% of pts, pregnancy for 6%, pt request for 62%, enrollment in ISAV protocol for 12%. After a median follow-up of 34 mos (IQR 23.5; 53.2) estimated TFR was 62% (95%CI 56; 67.6). Reasons for restarting were: loss of MR4 for 19% of pts, loss of MR3 for 69%, loss of CCyR for 9%, other reasons for 3%. Median time to restart treatment was 6 mos (IQR: 4; 11). We assessed age, sex, Sokal score, type of transcript, previous IFN therapy, duration of TKI therapy, response at 3 mos, time to CMR, CMR duration, line of therapy, depth of MR, reasons for stop as potential prognostic factors for TFR, but no statistically significant association were found, with the exception of age: a decreased risk in older *vs* younger pts. Pts who restarted therapy were treated with imatinib (77), nilotinib (22), dasatinib (9), bosutinib (3) or ponatinib (1). All of them regained at least MR3, with the exception of 7 not reported and 10 with no MMR. No pts progressed. Conclusion Although our study have the limitation of a restrospective study, our experience confirms that discontinuation of TKIs is feasible and safe in the clinical practice.

P085

TYROSINE KINASE INHIBITORS SIGNIFICANTLY CHANGE THE EXPRESSION OF POLYCOMB GENES IN CHRONIC MYELOID LEUKEMIA

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It has been reported that tyrosine kinase inhibitors (TKIs) are not able to eradicate the leukemic stem cell (LSC) in patients with chronic myeloid leukemia (CML). The mechanisms linked to the niche and the epigenetic control seems to be relevant, and our group previously identified a correlation between the expression of some polycomb genes (PcGs) and response to TKIs, with BMI1 resulting a good predictive molecular marker (Crea, 2015). To better understand the role of the PcGs, we analyzed the expression of 86 genes by real-time PCR (PrimePCR pathway kit, Biorad, Milan, Italy) at baseline and after 6 months of therapy of 9 patients (5 receiving imatinib, 3 nilotinib and 1 dasatinib) after quantification of the BCR-ABL1/ABL1 ratio%IS. Expression values were calculated by the Vandesompele method using four housekeeping genes. At the sixth month of treatment, 8 patients were in optimal response and one was "warning", according to the 2013 ELN guidelines. Among the tested genes, 69 resulted up-regulated, whereas 6% were down-regulated in almost one post-treatment samples. Among the up-regulated genes, some could be relevant from a biological point of view: HLTF, a target for RUNX1, whose low expression correlates with poor

outcome in acute leukemia; PHC2, able to silence the HOX genes, overcoming the multidrug resistance in myeloid models; PCGF5, a marker of normal hematopoiesis; MOV10, reported to have an anti-viral activity by increasing levels of gamma interferon. This is particularly interesting because it could explain our previous observation that Torque Teno virus replication does not occur in CML patients during TKIs therapy; SIRT1, whose up-regulation increases the oncogenic ability of K562 cells in a murine model. Among the down-regulated genes: CBX2, whose binding to P16/p19 promotes the cell cycle progression, and whose down-expression induces apoptosis; SMARCA1, correlated to the cell cycle progression; DNMT3B, whose high levels have been reported in stem cells, and whose reduction could characterize the differentiation process; ZBTB16, whose decrease could be a sign of reduced osteoblastogenesis in the niche. Finally, BMI1 levels resulted unmodified in 4 cases and increased in 5. We demonstrated that PcGs de-regulation occurs in CML patients during the treatment with TKIs, with possible pathogenetic implications. The analysis of a larger number of patients will improve the biological suggestions coming from these preliminary data.

P086

INFLAMMATION AND INCREASED ATHEROTHROMBOTIC RISK IN CHRONIC MYELOID LEUKEMIA PATIENTS DURING TKI TREATMENT: INTERIM ANALYSIS OF "KIARO" PROSPECTIVE MULTICENTER STUDY

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Background: Cardiovascular (CV) events, mostly atherothrombotic, in Chronic Myeloid Leukemia (CML) patients (pts) treated with Tyrosine Kinase inhibitors (TKIs) prompted physicians to evaluate CV risk factors (CVRFs) in the choice of TKI. However, the pathogenesis behind CV events during TKIs is still unknown and even pts without overt CVRFs incur in CV events. We retrospectively showed that an induced "inflammatory status" during nilotinib treatment, together with genetic pro-atherothrombotic predisposition, may explain the increased incidence of CV. These data provided the rationale for a multicentric study including CML pts treated with any first line TKI in which clinical, genetic and biochemical pro-atherothrombotic profiles were evaluated at diagnosis and during treatment (KIARO study). *Aims:* To evaluate prospectively the role of genetic predisposition and pro/anti-inflammatory factors in the atherosclerotic pathogenesis during TKIs. *Methods:* Pts were studied for: CVRFs, atherothrombotic events, Single Nucleotide Polymorphisms (SNPs) associated to CV risk and levels of pro/anti-inflammatory cytokines. In this interim analysis we focused on LDL, oxidated-LDL (oxLDL), TNF, IL6 and IL10 levels and SNPs of LDLR, LOX1, and IL10 genes. *Results:* Up to date, 115 CML pts from 16 Italian Hematology Units were enrolled. We report data from the first 43 pts on TKIs for at least 12 months (15nilotinib,14imatinib and 14dasatinib). No CV events were recorded to date. At diagnosis, LDL, oxLDL, TNF, IL6 and IL10 levels were evaluated. No differences were found between the 3 groups. We confirmed a correlation between LDL, oxLDL and IL10 basal levels with the presence/absence of their corresponding detrimental alleles. During TKIs we observed increased levels of LDL and oxLDL only in the nilotinib cohort at 3 and 12 months of treatment, regardless of the concomitant use of CV medications. No differences in TNF and IL6 levels during the first 12 months of TKIs were detected in the 3 cohort. IL10 levels were higher at 3 and 12 months of treatment in the imatinib and dasatinib cohort respect to nilotinib. *Discussions:* This interim analysis, although preliminary, suggests that in nilotinb pts the high levels of LDL and oxLDL in combination with low levels of IL10, may induce a pro-inflammatory/oxidative status potentially favoring atherothrombotic events. Additional biochemical and genetic data and prolonged clinical observation are needed to confirm this hypothesis.

P087

IMPACT OF AGE ON EFFICACY, SAFETY, AND LONG-TERM OUTCOME OF CHRONIC MYELOID LEUKEMIA (CML) PATIENTS TREATED IN FIRST-LINE WITH NILOTINIB: A GIMEMA CML WORKING PARTY ANALYSIS

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Background: In chronic phase (CP) chronic myeloid leukemia (CML) the disease characteristics at diagnosis, risk distribution, probability of transformation to accelerated/blast phase (AP/BP), and toxicities may vary according to age. Little is known on the efficacy, safety and long-term outcome of nilotinib treated patients (pts) in different age groups. *Aims:* To investigate the efficacy and safety, particularly the cardiovascular safety, of first-line treatment with nilotinib according to age distribution. *Methods:* We analyzed 345 pts ≥ 18 y of age with CP CML enrolled in clinical trials of the GIMEMA CML WP investigating nilotinib as first-line treatment. The median follow-up was 58 months. We divided the pts in 3 groups of age (table): 18-49 y (group A, 147 pts, median age: 39 y); 50-64 y (group B, 109 pts, median age 58 y); and ≥ 65 y (group C, 89 pts, median age 71 y). We analyzed in detail the response rates, events and 5-y outcome.

Table 1. Characteristics of patients.

	Group A (18 – 49 y)	Group B (50 – 64 y)	Group C (> 65 y)	
Patients, n	147	109	89	-
Median age, y	39	58	71	-
EUTOS High-risk, %	8.8	5.5	1.1	0.048
BCR-ABL/ABL <10% at 3 months, %	87	84	89	Ns
MMR at 12 months, %	57	57	54	Ns
MR4 at 12 months, %	30	32	25	Ns
MMR by 5 y, %	90	94	96	Ns
MR4 by 5 y, %	72	81	83	Ns
Progressions to AP/BP, %	6.8	1.8	1.1	0.037
Deaths, %	4.8	2.8	10.1	Ns
- Leukemia-related, %	100	100	11	-
- Leukemia-unrelated, %	0	0	89	-
6-year OS, %	94	97	84	0.12
Events	31	29	42	0.049
ATEs, % / per 1000 patient-y	2 / 5.3	11 / 26.8	15.7 /35.7	0.006
Permanent nilotinib D/C for ATEs	2	7.3	11.2	0.015

Results: EUTOS high-risk pts were 8.8, 5.5 and 1.1% in group A, B and C, respectively (p=0.048). We did not observe significant differences in molecular response rates (table), including BCR-ABL/ABL <10% at 3 months, MMR and MR4 at 1 year, and cumulative incidences by 5 y of MR3 and MR4. Events leading to permanent nilotinib discontinuations occurred in 31, 29 and 42% of pts in group A, B and C, respectively (p=0.049). Overall, 29 arterial thrombotic events (ATEs)

were recorded, with higher rates, as expected, in elderly pts (group A 2%; B 11%; C 15.7%; p=0.006). ATEs were the reason for permanent nilotinib discontinuation in 2% of pts 18-49 y, 7.3% of pts 50-64 y, and 11.2% of pts > 65 y (p=0.015). Progressions to AP/BP were significantly more frequent in younger (18 – 49 y) pts (6.8%) compared to older (> 50 y) ones (1.5%), p= 0.019. Overall, 19 pts died (group A: 4.8%; B: 2.8%; C: 10%). In pts < 65 y all deaths (10) were leukemia-related, while in pts >65 y, all but one (8/9) deaths were leukemia-unrelated. The 6-year overall-survival was 94%, 97%, and 84% in pts 18-49 y, 50-64 y, and > 65 y of age (p=0.12). *Conclusions:* Nilotinib as first-line treatment of newly diagnosed CP CML pts showed high rates of molecular responses in all age groups. However, while in pts > 50 y more attention should be focused on the prevention of ATEs, in younger ones more efforts are needed to avoid the progression of CML to accelerated/blast phase.

P088

THE TYPE OF TRANSCRIPT INFLUENCES THE PROBABILITY AND DURATION OF TREATMENT FREE REMISSION (TFR) AFTER BOTH IMATINIB AND NILOTINIB DISCONTINUATION IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS

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A substantial part of CML patients (pts) in deep molecular response (DMR) can safely stop TKI therapy. Sokal risk score and TKI duration influence the probability of molecular relapse, whereas few informations are available about the influence of BCR-ABL transcript type on TFR. We analyzed the outcome of 51 pts with CML who discontinued imatinib or nilotinib at our center. Criteria for TKI discontinuation was sustained DMR for at least 2 years. After TKI withdrawal, RQ-PCR was performed every month during the first year and every 2 months thereafter. TKI treatment was reintroduced if DMR loss occurred. Fifty-one pts discontinued TKI treatment: 37 pts stopped imatinib and 14 nilotinib. At time of discontinuation, median age was 63 (30-85), median time from TKI start 107 months (24-172) – 117 (37-172) for pts in imatinib and 50 (24-117) for pts in nilotinib- median sustained DMR 55 months (24-153)- 72 for imatinib and 37 for nilotinib-. Sokal distribution was 49%, 29% and 20% for low, intermediate and high risk (one patient was not evaluable). e14a2 transcript was expressed in 41 (80%) cases – 31 of them stopped imatinib and 10 nilotinib-, e13a2 transcript in 10 (20%) – 6 stopped imatinib, 4 nilotinib-. Median follow up from discontinuation was 21 months (1-78). DMR was lost in 13/51 (25%): recurrence rate was different in e14a2 and e13a2 pts, but, within each group, pts in imatinib and nilotinib had comparable relapses (6/31 e14a2-imatinib and 2/10 e14a2-nilotinib *vs* 3/6 e13a2- imatinib and 2/4 e13a2-nilotinib). Median time off-therapy for relapsed pts was 3 months (2-8). Therapy was restarted in all 13 pts, 11 achieved a second DMR, 2 are in MR3. Univariate analysis showed no difference in relapse risk according to age, gender, type and duration of TKI, duration of stable DMR and sokal score risk. Only the type of bcr-abl transcript significantly impacted DMR loss: after 12 months from TKI discontinuation, 80% of e14a2 and 50% of e13a2 pts were still in TFR (log-rank: P=0.03, hazard ratio 0.19, CI 0,04-0.85). At multivariate analysis the type of bcr-abl transcript remains a significant prognostic factor. After TKI discontinuation, pts expressing e14a2 transcript have significantly lower probability of DMR loss than e13a2 pts; in addition, the type of TKI does not influence the rate of recurrence within each group. The type of BCR-ABL transcript appears to predict TFR persistence after both imatinib and 2GTKI discontinuation in CML pts.

P089

FIRST-LINE TREATMENT WITH NILOTINIB IN CHRONIC MYELOID LEUKEMIA PATIENTS: RESULTS OF THE OBSERVATIONAL STUDY GIMEMA CML0912

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Introduction: Three tyrosine-kinase inhibitors (TKIs, imatinib, nilotinib[NIL] and dasatinib) are approved as first line treatment of CP CML. Clinical trials, with stringent limiting inclusion/exclusion criteria and management, may be not fully representative of the daily clinical practice. Thus, it is important to retrieve information on the safety and efficacy of TKIs in the real-life setting. Observational studies are particularly useful for these evaluations. *Aim:* To investigate the safety and efficacy of NIL as first line treatment of newly diagnosed CP CML pts in the real life setting. *Methods:* Prospective, multicenter, observational study. Inclusion criteria: CP CML within 6 months from diagnosis; no prior treatment with TKIs; written informed consent. Comorbidities (of any kind and severity) were not exclusion criteria. NIL was used according to prescribing information. There was no formal (in the protocol) indication for dose modifications, management of adverse events, and response monitoring. *Results:* Between 06/2013 and 11/2015, 123 pts were enrolled in 27 Italian Centers; median age: 51 yrs; Sokal high-risk pts: 19%; EUTOS high-risk pts: 7%. Median follow-up: 18 months (updated results will be presented). At the last contact, all but one pt were alive. 113 (89%) pts were still on study (on NIL treatment); in the remaining pts, NIL discontinuation occurred in: 9 pts for failure/suboptimal response, including 1 progression to blast phase; 2 pts for toxicity; 1 pt for a CML-unrelated death, 2 pts for other reasons. At the last contact, of evaluable pts with at least 12 months of follow-up, 78% and 43% of pts were in MR3 and MR4, respectively. *Conclusions:* These preliminary data in real-life CP CML pts treated with NIL front-line showed that: 1) despite the absence of exclusion criteria for comorbidities, the median age of enrolled pts (51 years) was similar to that reported in clinical trials, and inferior to the median age of CML pts, suggesting that other TKIs may have been preferred in older pts; 2) NIL use was not limited to intermediate or high-risk pts; 3) efficacy was similar to that reported in clinical trials, with very low rates of progression to advanced phase and high molecular response rates. Overall, these data suggest that NIL was successfully employed in relatively younger pts, regardless of the CML risk, perhaps with the aim of an early and sustained deep-molecular response, a prerequisite for treatment-free remission.

P090

THE EUTOS LONG-TERM SURVIVAL SCORING SYSTEM IS PREDICTIVE FOR RESPONSE AND OUTCOME IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED FRONTLINE WITH NILOTINIB

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Background: In the tyrosine kinase inhibitors (TKIs) era, many patients die of reasons unrelated to Chronic Myeloid Leukemia (CML). The new EUTOS long-term survival (ELTS) score, based on a large cohort of CML patients treated frontline with imatinib (IM) and aimed to discriminate the probability of dying of CML, has been recently published. Few data on the predictive value of ELTS score in CML patients treated frontline with 2nd generation TKIs are available. *Aims:* To assess the prognostic value of ELTS score in a cohort of CML patients in chronic phase treated with nilotinib (NIL) or NIL-based regimens as first-line therapy. *Methods:* The patients were enrolled in three multicenter studies (NCT00481052, NCT00769327, NCT01535391) or treated at the University Hospital of Bologna were analyzedy. The initial treatment was NIL 300 mg BID (43%) or NIL 400 mg BID (57%). The intention-to-treat population of each study was analyzed. *Results:* 345 adult patients were included; median age, 53 years (range 18-86 years). The patient distribution according the different scoring systems was as follows: 41% low, 40% intermediate and 19% high Sokal score; 59% low, 30% intermediate and 10% high ELTS score, respectively. Despite the variables included in the two scores are the same (age, blast percentage, spleen size and platelet count), discordances between the two scores have been observed: 92% of low Sokal score patients had a low ELTS score, while only 33% of high Sokal score patients had a high ELTS score. The median follow-up was 5.1 years. The cumulative incidence of Major Molecular Response (MMR) and Deep Molecular Response (MR4.0) was 83% and 69%, respectively; the 6-year overall survival (OS) and cumulative incidence of leukemia-related death (LRD) were 92% and 4%, respectively. Both Sokal and ELTS scores were associated with significantly different probabilities of MMR, but only the ELTS score was able to predict the achievement of MR4.0 (cumulative incidence of MR4.0: 75%, 63% and 53% in low, intermediate and high ELTS score patients, respectively; p=0.012). Both the Sokal score and the ELTS score, predicted the OS, while only the ELTS score predicted a significantly different LRD (estimated cumulative incidence of LRD: 2% and 6% in low risk and non-low risk patients, respectively; p=0.049). *Conclusions:* In a cohort of CML patients treated with NIL-based regimens as frontline therapy, the ELTS score resulted superior to Sokal score, predicting LRD (borderline significance), OS, MMR and MR4.0.

Chronic Myeloid Leukemia 2

P091

STANDARD DOSE (SD) *VERSUS* LOW DOSE (LD) OF IMATINIB IN A "REAL-LIFE" COHORT OF VERY ELDERLY PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA

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Background: In the "real world" of clinical practice, often physicians choose to treat very elderly CML patients with Imatinib (IM) at lower doses than standard, but there are no published data on the results. *Aims:* To assess toxicity, response and outcome of low-dose IM (≤300) *versus* standard dose in patient older than 75 yrs. *Methods:* A cohort of 263 CML patients aged >75 yrs and treated frontline with IM in 36 haematological Italian Institutions was retrospectively collected. The present analysis compared 83 patients (31.6%), who received ≤ 300 mg/day according to physician decision (LD group), with the remaining 180 patients (68.4%), who received the standard dose of 400 mg/day (SD group). *Results:* Median age at diagnosis was 77.9 yrs [interquartile range (IQR) 76.0-80.1] in the SD group *versus* (*vs*) 80.8 yrs (77.9-84.2) in the LD group (p<000.1). Gender, Sokal risk and median time from diagnosis to IM start were not significantly different. In the SD group 28 patients (15.6%) had ≥4 concomitant diseases *vs* 25 patients (30.3%) in the LD group (p=0.023): the frequency of diabetes, chronic renal insufficiency and chronic obstructive pulmonary diseases was significantly higher in the LD group. Grade 3-4 hematological toxicity in the SD group was 22.7% *vs* 19.2% in the LD group (p=0.52); grade 3-4 extra-hematological toxicity was 18.8% in the SD group *vs* 20.4% in the LD group (p=0.76) Overall, 97 patients (53.8%) in the SD group required a dose reduction compared to 25 (30.1%) in the LD group (p=0.004). Response to treatment is reported in the Table. After a median follow-up of 52.6 months (IQR 30.5–81.6), in the SD group 59 patients died (4 from disease progression and 55 from unrelated causes), 14 patients were lost to follow-up and 107 are still alive: in the LD group, 34 patients died (4 from disease progression and 30 from unrelated causes), 12 patients were lost at the follow-up and 37 are still alive. In the SD group, 2 and 5-year overall survival were 93.0% (CI95% 89.1–96.9) and 73.9% (CI95% 66.7–81.1), respectively; in the LD group, 2-year and 5-year overall survival were 93.4% (CI95% 87.9–98.9) and 63.5% (CI95% 51.1–75.9), respectively (p=0.007). *Conclusions:* Our data show that very elderly patients treated with LD IM can achieve therapeutic results similar to patients treated with SD, without different toxicity but with an inferior overall survival due to the higher rate of CML unrelated deaths among these older and frailer subjects.

Table 1. Response to treatment according starting Imatinib dose.

	SD group	LD group	*p*
N° patients evaluable for response	180	83	
Early discontinuation, n (%)	8 (4.4)	5 (6.0)	*0.583*
Resistant disease, n (%)	4 (2.2)	1 (1.2)	*0.574*
Complete haematological response only, n (%)	22 (12.2)	15 (18.0)	*0.205*
Partial cytogenetic response, n (%)	10 (5.5)	8 (9.6)	*0.223*
Complete cytogenetic response, n (%)	136 (75.7)	54 (65.2)	*0.070*
Major molecular response, n (%)	105 (58.3)	43 (51.8)	*0.321*
Evolved in Blastic Phase	7 (3.8%)	4 (4.8)	*0.472*

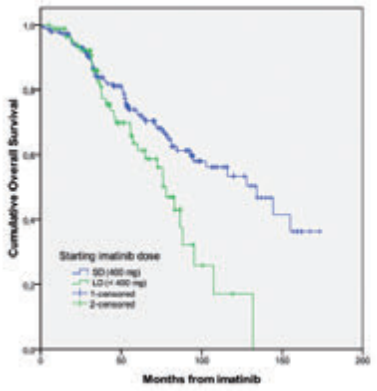

Figure 1. Overall Survival according to starting imatinib dose.

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LONG TERM FOLLOW-UP AFTER IMATINIB DISCONTINUATION IN CHRONIC MYELOID LEUKEMIA PATIENTS: AN OBSERVATIONAL CLINICAL TRIAL CARRIED OUT IN THE DEPARTMENT OF HEMATOLOGY, TRASFUSIONAL MEDICINE AND BIOTECHNOLOGY OF PESCARA

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Imatinib treatment significantly improves survival in pts with Chronic Myeloid Leukemia (CML). The STIM study demonstrated that Imatinib could be safely discontinuated in pts with deep molecular response (DMR), *i.e.* with undetectable minimal residual disease (UMRD) of at least 2 years. The TWISTER study demonstrated that around 40% of CML pts with stable DMR on Imatinib for at least 2 years are likely to remain in a prolonged treatment-free remission after treatment is stopped. We conducted an observational clinical trial of Imatinib withdrawal in 13 chronic-phase CML pts who had sustained UMRD by conventional quantitative polymerase chain reaction (RT-PCR) on Imatinib for at least 2 years. Patients which stopped Imatinib were monitored every month during the first year and every 2 months thereafter. The molecular relapse is defined as positivity of BCR-ABL transcript in quantitative RT-PCR. That analysis, which is confirmed by a second analysis point, indicates the increasing of one log in relation to the first analysis point, at two successive assessments, or loss of MMR at one point. In our study were recruited 13 pts (3 male, 10 female) with CML (all pts were previously treated with Hydroxyurea) and a median age of 58 years (range: 36-75). Considering the Sokal Risk Score, 11 patients were classified with a Low grade risk and 2 patients were classified with an Intermediate risk. The median follow-up from discontinuation in our study was 21 months (range: 4-49 months). Two pts relapsed after the discontinuation. The first patient, with an Intermediate risk, stopped Imatinib treatment after a continued therapy of 8 years and 3 months and the relapse occurred after 87 days. The second patient, with a Low-grade risk, stopped the treatment after a continued therapy of 15 years and 7 months and the relapse occurred after 128 days. The cumulative incidence (CI) of relapse was 15,4%. No pts relapsed after 6 months. All pts were sensitive to TKI re-challenge, according to the BCR-ABL transcript value and were re-treated with Imatinib. Imatinib can be safely discontinued in pts with a DMR of at least 2 years duration and we continue to recommend drug discontinuation only in clinical trials with closing molecular monitoring. Molecular relapse occurred during the first 4 months following Imatinib discontinuation and no MR was recorded after 4 months. The loss of MMR is a practical and safe criterion for restarting therapy in pts with CML with prolonged CMR.

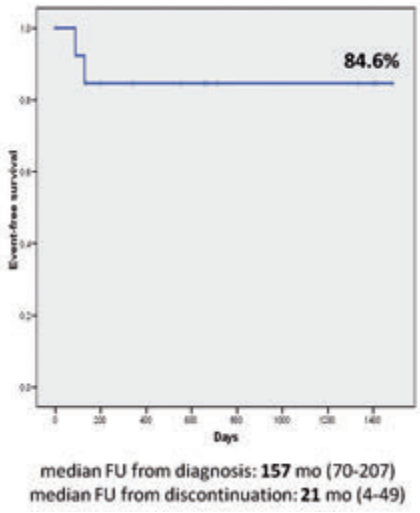

Figure 1. Overall Survival according to starting imatinib dose.

P093

BODY MASS INDEX DOES NOT IMPACT ON MOLECULAR RESPONSE RATE OF CHRONIC MYELOID LEUKEMIA (CML) TREATED FRONTLINE WITH SECOND GENERATION TKIS

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We previously reported that CML patients with increased BMI treated with imatinib witnessed a significantly longer median time to achieve a complete cytogenetic response (CCyR) and a reduced rate of major molecular response (MMR). We correlate the response rate in CML patients treated frontline with the second generation tyrosine kinase inhibitors (TKIs) to define whether the BMI could be a prognostic factor to be considered at baseline. BMI was defined as the body mass divided by the square of the body height, universally expressed in Kg/m2. According to the BMI, patients were classified into 4 subsets: underweight (<18.5), normal weight (>18.5-<25), overweight (>25-<30) and obese (>30). Seventy-eight patients diagnosed from May 2007 to October 2016 and treated frontline with nilotinib (53 patients) or dasatinib (25 patients) entered this analysis. Forty patients (51.3%) were categorized as underweight/normal weight, while 38 (48.7%) were classified as overweight/obese. Median age at diagnosis was 56 and 55 years for the two subsets of patients, respectively (p=ns). A correlation was identified between increased BMI and gender, with a higher percentage of males being overweight/obese compared to females (68% *vs* 42%; p=0.01). We found no significant association between BMI and prognostic risk stratifications at diagnosis (Eutos, Sokal, Hasford and ELTS). At 3 months, there were no significant differences between patients with low BMI (<25) and high BMI (>25) in terms of CCyR rate (97.5% *vs* 100%, p=0.32), early molecular response (92.4% *vs* 94.7%, p=0.68) and MMR rate (52.5% *vs* 42.1%, p=035). The results were similar at 12 months of treatment with MMR being 75.7% *vs* 85.3% (p=0.32) and MR4-4.5 45.4% *vs* 50% (p=0.50). Finally, we assessed the cumulative incidence of molecular responses achieved after a median follow-up of 40 months. Again, no differences were found in terms of MMR rate (35% *vs* 28.9% p=0.56), MR4.0 (22.5% *vs* 28.9%, p=0.51) and MR4.5 (30% *vs* 23.7%, p=0.62) for underweight/normal weight and overweight/obese patients, respectively. Our results suggest that BMI assessment should be considered as a baseline factor before starting any type of treatment. Considering treatment free-remission as a new endopoint, imatinib seems contraindicated as frontline treatment for obese patients. Indeed, obesity seems an initial risk factor counterbalanced by the use of second generation TKIs, even if the cardiovascular risk and other comorbidities should be taken into account in this subset of patients.

P094

CHRONIC MYELOID LEUKEMIA TREATMENT: NO NEED FOR HYDROXYUREA CYTOREDUCTIVE PRE-TREATMENT AND EVIDENCE OF QUANTITATIVE CHANGES IN THE EXPRESSION OF THE BCR-ABL1 TRANSCRIPT DURING THE FIRST TWO WEEKS OF THERAPY

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Pre-treatment with HU is usually administered to CML patients with high WBC counts before starting any TKIs therapy. Up to date there is no evidence of any advantage of HU pre-treatment, even in patients with high WBC-counts; conversely recent data supports both the prognostic significance of the quantitative assessment of BCR-ABL expression at diagnosis and the individual decline of the BCR-ABL1 slope. We speculated that the amount of BCR-ABL transcripts at diagnosis may be indicative of the CML burden at the molecular stage. We also assumed that any kind of treatment administered before a confirmed diagnosis of CML might change the amount of BCR-ABL1 expression. With this aim, we evaluated WBC counts and BCR-ABL1 quantitative expression either at diagnosis and at day 7 and 14 of treatment in a cohort of 45 unselected adult patients with newly diagnosed BCR-ABL1-positive CP-CML. After informed consent, 21 of them received preliminary therapy with HU before starting TKIs treatment (HU-dataset), whereas the other 24 patients received frontline TKIs-therapy without any HU-therapy (TKIs-dataset). Hematological responses and BCR-ABL1 transcripts were measured from peripheral blood samples drawn at the above prefixed time-points. All RQ-PCR determinations were centralized and BCR-ABL1 values were converted to the International Scale using GUS as a reference gene. CML patients in the HU-dataset exhibited higher WBC-counts at diagnosis than those in the TKIs-dataset (WBC: 141175/mmc *vs* 86015/mmc; p=0.006), however, when we looked at the cytoreductive efficacy performing ANOVA analysis we do not detect any statistical difference at day 14 of therapy between the HU-dataset and the TKIs-dataset (WBC:41114/mmc *vs* 29793/mmc). At the same time-points we also found evidence of notable changes in the expression of the BCR-ABL1 transcripts among groups (HU-dataset: dx17.58IS; 14.73IS; 24.58IS *vs* TKIs-dataset: dx25.55IS; 24.00IS; 20.39IS) depending also on kind of therapy (HU *vs* TKIs). In fact TKIs-therapy showed a clear decline of the BCR-ABL1 transcripts whereas HU did not, suggesting that HU-therapy displays a minor impact against CML clones with high BCR-ABL levels. We also show that HU treatment administered before a confirmed diagnosis of CML changes the amount of BCR-ABL1 expression In conclusion we provide evidence that there is no need for HU cytoreduction in CML and that quantitative changes in BCR-ABL1 expression are differently driven by kinds of therapy.

P095

EFFICACY AND SAFETY OF SECOND LINE PONATINIB IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS

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Background: Scarce information is available on the use of ponatinib, a third-generation tyrosine kinase inhibitor (TKI), second line after resistance and/or intolerance to a single line of therapy in chronic phase chronic myeloid leukemia patients (CP-CML). In the PACE study, only 19 patients were described with an increased rate of response compared to patients who received the drug as third or fourth line of treatment, but no details about baseline features have been reported. *Aim:* We collected data related to the use of second line ponatinb in CP-CML patients in order to report efficacy and safety outside of a clinical trial, with a particular attention to the incidence of cardiovascular events. *Results:* Twenty-two patients were collected with a median age of 54 years (range 32-72). According to Sokal score, 14% of patients were classified as low risk, 59% as intermediate and 27% as high risk. Ten patients received first line dasatinib, 9 nilotinib and 3 patients were treated with imatinib. Forty-one% of patients started ponatinib for secondary resistance, 36% for primary resistance, 14% for severe intolerance associated to a molecular warning and 9% for T315I mutation. Ponatinib was started at the dose of 45 mg in 46% of patients, 30 mg in 45% and 15 mg in 9% of patients. As best response, with a median follow-up of 12 months (range 3-24), 1 patient achieved a PCyR and then underwent an allogeneic stem cell transplant because of an increasing molecular signal, 1 patient (primary resistant with a F359V mutation) a MCyR, 4 patients a CCyR (2 after 3 months and 2 after 6 months), 8 patients a MMR (5/8 patients treated with 30 mg), 4 patients a deep molecular response (MR4-4.5, including 2 patients with T315I). At 3 months, 11/22 patients did not achieve a BCR/ABL1 ratio <10%. With regards to safety, no patient experienced vascular thrombotic events: the more common side effects recorded were increased lipase (2 patients), hypertension (3 patients), skin rash (5 patients). The dose was reduced during treatment due to intolerance in 2 patients and due to achievement of response in 8 patients in order to reduce the cardiovascular risk. One patient progressed but none died during treatment. Overall, 85% of treated patients improved the level of response as compared to baseline. *Conclusions:* Ponatinib seems a valid second line treatment option for CP-CML, in particular for patients who failed a frontline second generation TKI due to BCR-ABL independent mechanisms of resistance. A longer follow-up and a larger cohort of patients are needed to confirm these results.

P096

EFFICACY AND SAFETY OF PONATINIB USED IN FRAIL CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS OUTSIDE OF CLINICAL TRIALS

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Background: The clinical use of ponatinib is complicated by the possible development of cardiovascular events: for this reason it is recommended to select patients and to monitor possible vascular side effects. *Aim:* We collected data related the use of ponatinb in frail CP-CML patients in order to detect the incidence of cardiovascular events, the compliance to the drug and efficacy. Participant physicians detailed the definition of "frailty" and the reasons for which the drug was used. *Results:* Twenty-seven patients were collected, median age 68 years (17 males and 10 females). Frailty condition was defined as advanced age (8 patients >75 years), coexistence of serious cardiovascular risk factors in 15 patients, psychiatric disorders in 3 patients, severe autoimmune disease in 1 patient. According to Sokal's score, 4 patients were low, 13 intermediate and 10 patients high risk. SCORE chart identified 18 patients as moderate/high risk. Charlson comorbidity index stratified 7 patients with a score higher than 5. Ponatinib was used as second line in 2 dasatinib-treated patients after pulmonary arterial hypertension and recurrent pleural effusion; as third line in 7 patients (all treated with dasatinib second line) and 18 patients treated as fourth line. Ten patients

presented mutations at baseline (4 T315I). Thirteen patients started with 45 mg, 8 patients with 30 mg and 5 patients with 15 mg. As best response, after a median follow-up of 12 months, 4/5 patients who started without a complete hematologic response (CHR, 1 with T315I) reached a complete cytogenetic remission (CCyR), with a MR4 in 1. Twelve patients started in CHR and 4 achieved a CCyR (2 patients T315I), 5 patients a MR3 (1 T315I), 1 patient a MR4, 2 patients did not reach any improvement. Nine patients were treated with a baseline transcript level >0.1% IS and 5 reached a MR3, while 4 patients achieved a MR4. Seven patients experienced cardiovascular side effects (cardiac ischemia in 3, cerebrovascular ischemia in 1, PAOD in 3): all patients had been previously treated with nilotinib and dasatinib, and all patients had >3 baseline cardiovascular risk factors (only 2 were in treatment with anti-aggregant prophylaxis). Four patients remained on treatment after the occurrence of the cardiovascular event. Hypertension was recorded in 5 patients, all with moderate borderline pressure at baseline. *Conclusions:* Ponatinib was manageable and effective in patients considered frail with limited cardiovascular toxicity. In the majority of patients, the dose was adjusted and then reduced after the achievement of a response.

P097

EFFICACY AND SAFETY OF NILOTINIB AS FRONT LINE TREATMENT IN A COHORT OF ELDERLY (>65Y)CHRONIC MYELOID LEUKEMIA PATIENTS IN REAL LIFE

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Background: Few data onefficacy and safety of second generation TKIs in first line treatment in elderly chronic myeloid leukemia (CML) patientshave been reported so far. Generally these patients are excluded by company sponsored trialsdue to the impact of comorbidities at baseline. *Aim:* A retrospective analysis of efficacy and safety of frontline nilotinib in elderly patients (>65 years old), collected in 18 Italian centers, is reported. *Materials and Methods:* 55 elderly CMLin chronic phase were enrolled (median age 72 yrs, M/F 28/27); 13 patients were older than 75 yrs. 50patients showed comorbidities: Charlson Comorbidity Index (CCI) was in median between 0-3, in 10 was 4-5. Concomitant medications wererequired in 43 patients. The ECOG score was 0 in the majority. The SOKAL score was high in 16, intermediate in 36 and low in only 3.34 patients had b3/a2 transcript, 5 coexpressed both, the remaining had b2/a2. *Results:* At 3 months, all patients were in complete hematological response, 43/55 in complete cytogenetic response (CCyR) too. 43 patients were in Early Molecular Response (EMR). The molecular results at different time point are summarized in Table 1. The majority of patients (85%) started therapy at 300 mg BID, 3 received 800 mg BID and 2 patients 300mg/die.10 patients showedG2 hematological toxicities (6anemia, 1 piastrinopenia, 2 both, 1 leucopenia);22 showed non hematological side effects, mostly ofG2 (11 dermatological,2 gastrointestinal,2 epatic,1 uncontrolled diabetesand 2 hypercolesterolemia).All these effects led to a dose reduction in 8patients.5 patients had cardiovascular events (1arrythmia, 1 atrial fibrillation, 1 left ventricular hypertrophy, 1 prolonged QTC, 1 not specified cardiotoxicity); four of these patients switched to other TKIs, all in MMR or deep molecular response.Ata median follow up of 46,29 months, 8 patients discontinued definitively the treatment for side effects, 3 patients died not for CML ;33 patient continued the therapy at the standard dose, 11 at lower dose. *Conclusions:* Our data shown the efficacy and safety profile of Nilotinib as first line treatment in elderly CML patients.The presence of comorbidities in higher proportion in this kind of patients didn't affect the molecular response and the tolerability. It could be possible in the long term treatment to adapt the dosing scheme to improve the tolerability, while maintaining the optimal molecular response.

Table 1. Molecular response (according to ELN recommendations).

Time (mo)	3	6	12	18	24
Patients (n)	55	46	41	40	33
EMR	**76,4%**				
No EMR	9,1				
MR3	12,7 %	37,0%	39,0%	37,5%	21,2%
MR4	3,6 %	17,4%	24,4%	17,5%	30,3%
MR4.5	1,8 %	0,0%	7,3%	17,5%	15,2%
MR5	0	0,0%	0,0%	7,5%	12,1%
No MMR		43,5%	19,5%	15,0%	12,1%
NE	14,5 %	2,1%	9,7%	5,0%	9,1%

P098

DASATINIB FRONTLINE IN ELDERLY PATIENTS WITH CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA

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Older age and comorbidities are often considered as key exclusion criteria from clinical trials in CML. However, in the real daily clinical practice, these features play a major role in the management of the elder CML patient. Dasatinib (DAS) has been licensed for first line treatment of patients (pts) with chronic myeloid leukemia (CML) and very few data are available in elderly CML pts as to toxicity and efficacy. To address this issue, we evaluated a "real-life" cohort of 72 CML pts in chronic phase aged >65 years and treated with frontline DAS in 26 Ital-

ian Centers from 6/2012 to 10/2016. Main clinical features at diagnosis are reported in the Table 1. According to Sokal they were stratified as follows: 4 patients (5.5%) were low risk, 43 (59.7%) intermediate risk, 22 (30.6%) high risk, while 3 (4.2%) were not classifiable. 41/72 pts (56.9%) had ≥ 2 comorbidities requiring concomitant therapies and performance status at baseline was 0–1 in 61 pts (84.7%) and 2 in 11 pts (15.3%) as to ECOG scale. Median interval from diagnosis to DAS therapy was 23 days (IQR 14–32). Dasatinib starting dose was 100 mg/day in 61 pts (84.7%) and <100mg/day in 11 pts (15.3%), respectively. All pts were evaluable for toxicity; on the whole, grade 3/4 hematological and extra-hematological toxicities were detected in 8 (11.1%) and 12 (16.7%) pts, respectively. DAS therapy was permanently discontinued in 12 pts (16.6%) due to toxicity (6 pts in the first 12-month period of treatment and 6 beyond). Pleural effusions of all WHO grades occurred in 14 pts (19.4%) after a median period of DAS treatment of 3.6 months (IQR 1.4–13.1): in 5 of them pleural effusion occurred during the first 3-month. Overall, 66/72 patients (91.6%) achieved complete cytogenetic response (CCyR), 55/72 (76.4%) a major molecular response (MR 3) and 29/72 (40.3%) a deep molecular response (MR 4.0/4.5). After a median period of 27,8 months (IQR 19.1–37.5), 7 patients died (1 from blastic phase and 6 from unrelated causes): cumulative event-free survival and overall survival at 24 months were 73.7% (95%CI 62.7–84.7) and 90.4% (95%CI 82.9–97.9), respectively. In conclusion, these data shows that DAS therapy might have a major role in the treatment of unselected patients aged >65 years, being effective and having a favourable safety profile, also in subjects with comorbidities.

Table 1. Patients features at diagnosis.

N° of patients	72
Males, N° (%)	36 (50.0)
Median age, years (interquartile range)	74.6 (68.8 – 78.7)
Median Hb value, g/dl (interquartile range)	12.6 (11.5 – 13.8)
Median WBC value, x 10^9/l (interquartile range)	42.5 (26.4 – 81.5)
Median PLT value, x 10^9/l (interquartile range)	456 (252 – 797)
Sokal risk: N° (%)	
Low	4 (5.5)
Intermediate	43 (59.7)
High	22 (30.6)
Not evaluable	3 (4.2)
Performance Status (ECOG), N° (%)	
grade 0 - 1	61 (84.7)
grade ≥2	11 (15.3)
Comorbidities requiring treatment, N° (%)	
0 – 1	31 (43.1)
≥ 2	41 (56.9)

P099

ROLE OF THE AURORA KINASE A/PLK 1 AXIS INHIBITION IN RESTORATION OF CELL GROWTH CONTROL OF CHRONIC MYELOID LEUKEMIA PROGENITORS

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Cell response to stress is a central component of genomic stability. The integrity of signaling pathways involved in cell cycle arrest, chromatin remodeling and DNA repair, are critical for the maintenance of replicated DNA fidelity. Gadd45 proteins function as stress sensors and gene transcription regulators. Gadd45a intervenes in G2/M checkpoint induction and DNA repair, and is required to prevent abnormal mitosis and aneuploidy. Such evidences lets assume a role of Gadd45a in cancer development and progression. Gadd45a interacts with Aurora Kinase A (AKA), a member of a serine-threonine kinase family active during mitosis and frequently overexpressed in human cancers where correlates with a poor prognosis. Notably, AKA overexpression is always associated with defects in centrosome duplication and aneuploidy, suggesting that it may enhance other oncogenic events by promoting genomic instability, one major trait of chronic myeloid leukemia (CML). Our results support the hypothesis that AKA cooperates with the constitutive TK activity of Bcr-Abl fusion protein by increasing DNA damage, promoting the occurrence of additional genomic alterations and driving TKIs resistance and disease progression to blast crisis. Here we investigated AKA and Plk1 role in CML hematopoietic progenitor survival as potential targets to eradicate the transformed clone. Preliminary experiments were aimed to determine whether IM resistance in a BCR-ABL1 cell context is associated with the over-expression and hyper-activation of AKA/PLK1 axis. In IM-resistant K562 cell line drug resistance was associated with increased expression and activation of AKA and Plk1. 24h exposure to IM significantly reduced expression and phosphorylation of both proteins in parental K562, but not in IM-resistant K562, indicating that AKA and Plk1 activation is only partly dependent on BCR-ABL1 TK activity. Subsequent experiments showed that the inhibition of AKA and Plk1 in response to specific inhibitors (Danusertib and Volasertib respectively) was associated with: significant increase of gadd45 a expression levels; reduction of cell survival; G2/M checkpoint arrest. The findings support the role of AKA/Plk1 inhibition in restoration of signals involved cell growth control and apoptosis. The advantage of using AK and Plk1 inhibitors in CML therapy mostly arises from effects independent from TK activity of Bcr-Abl protein. We proved that the AK and Plk1 inhibitors induce growth arrest and apoptosis in IM sensitive and resistant cell lines.

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WT1 IN CHRONIC MYELOID LEUKEMIA

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Introduction: Monitoring of CML patients is based on bcr/abl evaluation by RQ-PCR. ELN guidelines show that bcr/abl inferior to 10% after 3 months of therapy and inferior to 1% after 6 months is an optimal response. Different clinical parameters are used to predict patients' prognosis at onset in the 3 most commonly used score indexes. No other useful tools have been identified. WT1 gene is over-expressed at onset in several diseases including Acute Myeloid Leukaemia (AML):gene's levels decrease during remission and are low in healthy people. WT1 is over expressed in AML relapse. Few data are available on WT1 in CML patients. *Methods:* We evaluated 43 patients with CML referred to our centres between 2009 and 2017. RQ-PCR was performed to quantify bcr/abl and WT1 in the peripheral blood of patients at onset and after 3, 6, 9 and 12 months of therapy (median follow up 30 months, range: 9-96). Bcr/abl transcript was b2a2 in 10 pts, b3a2 in 27, both in 2, 4 were not analyzed. Sokal risk was low in 19 patients, intermediate in 15, high in 9. 23 patients received Imatinib, 6 Dasatinib and 14 Nilotinib as first line therapy. *Results:* 11/43 of patients (26%) had high levels of WT1 (WT1-H) at onset.Sokal risk was low in 3, intermediate in 4, high in 4. 32/43 of patients had low levels of WT1 (WT1-L): Sokal risk was low in 16, intermediate in 11, high in 5. 9/11 WT1-H patients were evaluable for bcr/abl at 3 months of therapy: 1 had bcr/abl >10%, 6 between 1 and 10% and 2 <1%. 29/32 WT1-L patients were evaluable at 3 months: 4 had bcr/abl >10%, 7 between 1 and 10%, 18<1%.At 6 months the differences between the 2 cohorts seems clear: 55% of WT1-H and 10% of WT1-L patients had bcr/abl levels >1%. The outcome of the groups seems different:during follow up 7/11 WT1-H patients had a failure according to ELN criteria and needed to change therapy.Only 2/32 WT1-L patients had a failure and underwent a second line therapy. We observed differences in the quality of response between the two groups, too. 5/11 WT1-H patients actually are in MR4, 2 patients in MR3, 2 are in failure and 1 patient died. 20/32 WT1-L patients are in MR4, 9 in MR3, 2 MR2, 1 in failure. *Conclusions:* In our cohort, WT1-H patients seem to have a worse outcome than WT1-L, in terms of MR4 and shift to second line therapy due to failure: 64% of WT1-H patients *vs* 6% of WT1-L patients needed to shift

to a different TKi.WT1 could be a prognostic marker at onset, but we need to study a larger number of patients to confirm this hypothesis.

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IS ERYTHROPOIETIN THERAPY USEFUL IN SEVERE (HB ≤10G/DL) CHRONIC ANEMIA OF ELDERLY PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB?

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Background: Severe late chronic anemia was reported in a sizeable rate of patients with Chronic Myeloid Leukemia (CML) responsive to imatinib and seems to be more common in elderly subjects: the role of erythropoietin (EPO) to treat this late effect is still unknown. *Methods:* To highlight this issue, we revised retrospectively 81 CML patients aged >60 years treated at our Institution with frontline imatinib for at least 24 months who achieved a durable complete cytogenetic response (CCyR). Severe late chronic anemia was defined as the presence of persistent (>6 months) and otherwise unexplained (creatinine level <2mg/dl, normal iron balance, bilirubin level <2mg/dl, folate and Vitamin B12 in the normal range) Hb levels ≤10g/dl after >6 months from imatinib start. *Results:* A condition of late severe chronic anemia occurred in 22/81 patients (27.2%) at different intervals from imatinib start. Seven out 22 patients (31.8%) needed packed red cell transfusions during the follow-up. Clinical features at diagnosis of patients who had severe late chronic anemia compared to the remaining 59 patients without anemia are shown in the Table 1. Among the 22 patients with severe late chronic anemia, 6 were treated with subcutaneous recombinant alpha-EPO at the standard dosage of 40,000 UI weekly, after a median time from imatinib start of 44.7 months (absolute range 26.8–73.8): in all 6 patients, baseline endogenous EPO levels were <100mUI/ml. All 6 patients achieved erythroid response, which was complete (achievement of stable Hb levels >12g/dl) in 4 patients and partial (stable increment >1.5g/dl of Hb levels with Hb levels <12g/dl) in 2 patients: one patient had a relapse of anemia after 24.1 months from EPO start and stopped EPO treatment, the remaining 5 patients are still in response and in treatment with EPO. No EPO-related toxicity was observed. Cumulative 4-year Event-Free Survival (EFS) for patients with severe late chronic anemia was 69.7% (CI95% 49.3–90.1) compared to 86.2% (CI95% 77.4–95.0) for patients without anemia (p=0.075). *Conclusions:* Severe chronic anemia during long-lasting treatment with imatinib has been observed in >25% of our responsive elderly patients, its occurrence seems more common in very elderly patients with lower Hb levels at diagnosis. Results with EPO are encouraging, but larger studies are warranted to define the role of such an approach in treating this common late complication of prolonged imatinib therapy

Table 1. Main features at diagnosis of patients with and without late chronic anemia in the follow-up.

	LATE ANEMIA	NO LATE ANEMIA	p
M/F	12/10	30/29	0.767
Median age (yrs) (IR)	74.1 (68.1 – 80.0)	69.8 (63.9 – 75.2)	**0.036**
Sokal Score: Low	2	10	
Int	16	42	0.584
High	4	7	
Median Hb (g/dl) (IR)	11.5 (10.9 – 13.4)	12.9 (12.1 – 13.8)	**0.030**
Median WBC (x 10^9/l) (IR)	41.0 (26.2 – 71.0)	43.4 (25.5 – 80.5)	0.958
Median PLTS (x 10^9/l) (IR)	421 (271 – 763)	456 (286 – 676)	0.857

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FRONT-LINE TREATMENT OF BCR-ABL+ CHRONIC MYELOID LEUKEMIA WITH DASATINIB: A PROSPECTIVE MULTICENTRIC OBSERVATIONAL STUDY

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Background: Dasatinib (DAS) is a 2nd generation tyrosine kinase inhibitor (TKI) approved for 1st line treatment of BCR-ABL+ Chronic Myeloid Leukemia (CML). Most data on 2nd generation TKIs are from company-sponsored studies, but the patient selection and the high rate of study discontinuation may jeopardize data interpretation. A post-marketing surveillance in large independent trials is extremely important. *Aims:* To describe, in the clinical practice, the response to DAS and the events leading to permanent treatment discontinuation. *Methods:* An italian multicentric prospective observational study of CML patients treated frontline with DAS has been conducted by the GIMEMA CML Working Party. DAS was given at the discretion of investigators, according to prescribing information, without any exclusion criteria, apart from age (<18 years old) and prior treatment with TKIs or interferon. Molecular response was assessed in standardized molecular laboratories (Labnet network). *Results:* 147 CML patients in early chronic phase have been enrolled. The median age was 57 years (65 years or older: 34% of patients). High risk patients according to Sokal, Euro and EUTOS score were 28%, 11%, and 13%, respectively. Median follow-up: 12 months. At 3 months, 88% of patients had BCR-ABL transcript levels <10% (at the same timeopoint, transcript <1% in 66% of patients). At 6 months, 81% of patients had BCR-ABL transcript levels <1%. The major molecular response rates at 6 and 12 months were 53% and 72%, respectively. The proportion of patients achieving a MR4 or a MR4.5 at least once was 35% and 16%, respectively. At the last contact, the patients still on treatment with DAS were 88%, while 12% permanently interrupted the study drug: 1% progression, 2% failure, 6% adverse events (pleural effusion and hematologic toxicity were the most frequent events leading to treatment discontinuation), 1% lost to follow-up, 1% unrelated death, 1% other reasons. *Conclusions:* The median age of patients receiving DAS in our trial was close to population-based CML registries, with one third of elderly patients (at least 65 years old). The proportion of high risk patient according to all scores was even greater. However, high molecular response rates were observed. The most frequent reason of permanent treatment discontinuation was the occurrence of adverse events.

Myeloproliferative Syndromes 1

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OPTIMAL TIMING OF ASPIRIN INTAKE IN ESSENTIAL THROMOCUYTHEMIA

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The essential thrombocythemia (ET) is a myeloid neoplasm characterized by platelet hyperreactivity and thrombotic risk. The treatment with aspirin (ASA) is recommended in ET patients at risk of first-time or recurrent thrombotic events. An unexplored topic is the optimal timing of once daily ASA intake. On the basis of the presumptions that 1) platelet aggregation is higher in the morning and that 2) the platelet inhibitory effect of ASA is not sustained during the usual 24-hour (h) dosing interval and that 3) a higher gastric mucosal resistance in the evening, we evaluated platelet count, -thromboglobulin (-TG) and platelet factor 4 (PF4), as markers of platelet activation, the platelet function activity (PFA), as indicator of ASA platelet sensitivity, the clotting time (CT), as indicator of aspirinated platelet contribution to clot formation. We studied 60 patients (20 men, 40 women; mean age 51 years, range 32-70) with ET according to WHO criteria. The mean duration of disease was 11 years. All patients were on ASA 100 mg once daily. Of these, 30 took ASA on awakening and 30 took ASA at bedtime. Of the 60 patients, 45 were on anagrelide hydrochloride (daily dose 1.5 mg) (10 men, 35 women), 15 were on hydroxyurea (daily dose 2 mg) (10 men 5 women). None had inherited or acquired thrombotic risk factors. Sixty subjects served as controls. Platelets were measured by automated analyzer. -TG and PF4 were determined by ELISA. ASA platelet sensitivity and CT were measured by Platelet Function Analyzer (PFA-100) and by ROTEM delta, respectively. The mean platelet count was 455±200x109/L. The awakening ASA patients had normal -TG and PF4 (12±5 IU/ml and 4±1 IU/ml), prolonged C/EPI closure time (T, unit: s, n.v. 84-160 s) (249±40 s) and normal CT (CT, unit: s. n.v. 100-240 s) (110±20 s), whereas the bedtime ASA patients had high -TG and PF4 (200±15 IU/ml *vs* 20±11 IU/ml and 170±50 IU/ml *vs* 6±2 IU/ml, respectively) (p<.0001 and p<.0001, respectively), normal C/EPI closure time (T, unit: s, n.v. 84-160 s) (90±10 s) and shortened CT (CT, unit: s. n.v. 100-240 s) (80±10 s). These findings suggest that in ET patients the optimal timing of once daily ASA intake is in the morning.

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COMPARISON OF EFFICACY AND SAFETY OF PEGYLATED INTERFERON -2B AND -2A FOR THE TREATMENT OF ESSENTIAL THROMBOCYTHEMIA: A MONOCENTRIC EXPERIENCE

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Background: Intereferon-alfa is an effective therapy in controlling platelet count in patients with Essential Thrombocythemia (ET), even if it is associated with significant adverse events and a discontinuation rate of above 20%. Some studies demonstrated that pegylated interferon -2b and -2a in ET (PEG-IFN -2b and PEG-IFN -2a) has a superior pharmacokinetic and toxicity profile compared with IFN-. *Aims:* The aim of our study was to compare the efficacy and toxicity profile of two formulations of PEG-IFN for the treatment of ET patients. *Methods:* Patients with ET treated with PEG-IFN -2b subcutaneously with a starting dose of 50µg weekly, or with PEG-IFN -2a, subcutaneously at starting dose of 135µg weekly were identified at our Institution. Therapeutic response was calculated by the revised ELN/IWG-MRT criteria including complete remission (CR), partial remission (PR) and no response (NR). Toxicity to therapy was assessed using the CTCAE 4.0 criteria. *Results:* From January 2003 to January 2017, fifty patients received PEG-IFN -2b, whereas 23 patients received PEG-IFN -2a. The median follow-up time was 41.3 months for the PEG-IFN -2b group *vs* 26 months for the PEG-IFN -2a group. A total of 37 (74%) patients in the PEG-IFN -2b group achieved CR, as compared with 19 (82.6%) in the PEG-IFN -2a group (p=0.42), the median time to achieve CR was 1 months for both groups. PR rate was slightly higher in the PEG-IFN -2b group compared with the PEG-IFN -2a group (22% *vs* 4.3%, p=0.06), NR rate was similar (PEG-IFN -2b 4% *vs* PEG-IFN -2a 11%, p=0.15). Eleven patients who received PEG-IFN -2b (20%) and one patient who received PEGIFN- -2a (4.3%) discontinued the treatment because of flu like symptoms, p=0.06. All grades nonhematologic adverse events occurred more frequently in the PEG-IFN -2b group compared with PEG-IFN -2a group: flu like symptoms (72% *vs* 39.1%, p=0.007), fatigue (60% *vs* 13%, p<0.001), injection site reaction (36% *vs* 4.3%, p=0.004) and autoimmune disorders (20% *vs* 4.3%, p=0.08), (predominantly grade 1 or 2). Lymphopenia, neutropenia and anemia were the most frequent hematologic adverse events (predominantly grade 1 or 2), and in no case they have represented a reason for treatment discontinuation. *Conclusions:* Our data confirmed the efficacy and safety of both PEG-IFN for ET treatment. No difference was found regarding the CR rate between the two formulation. PEG-IFN -2a showed a better tolerability profile compared with PEG-IFN -2b, leading to a low discontinuation rate due to adverse events.

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EFFICACY AND SAFETY OF RUXOLITINIB IN ELDERLY PATIENTS WITH MYELOFIBROSIS: A MULTICENTER SURVEY

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Ruxolitinib (RUX) is a JAK1/2 inhibitor that may control myelofibrosis (MF)-related splenomegaly and systemic symptoms. Although RUX may be prescribed regardless of age, elderly pts are underrepresented in clinical trials and the role of RUX in this population is relatively unknown. We report on 277 pts with WHO-defined MF treated with RUX when aged ≥65yrs. Charlson Comorbidity Index (CCI) and Body Mass Index (BMI) were evaluated at RUX start. Spleen (SR) and Symptoms (SyR) Response to RUX were evaluated according to the 2013 IWG-MRT criteria. Compared to elderly (age 65-74) pts, very elderly (≥75yr) pts had a higher CCI and a lower platelet count, thus starting RUX with lower doses (Table 1). However, average RUX doses during the first 3 mos were comparable in the two cohorts (p=0.13). At 6 months, SR and SyR were achieved by 35.9% and 83.7% of evaluable pts, respectively. Responses were comparable in elderly and very elderly pts (SR: 34.8% *vs* 36.6%, p=0.88; SyR: 80.7% *vs* 85.7%, p=0.35). A high (20mg BID) RUX starting/titrated dose during the first 12 weeks was significantly associated with SR at 6 months. At 3 months, drug-related anemia/thrombocytopenia ≥G2 were observed in 45.5% and 34.6% of evaluable pts, respectively. The incidence of anemia/thrombocytopenia was not influenced by age (anemia: 62% *vs* 70%, p=0.19; thrombocytopenia: 10% *vs* 18%, p=0.07). 97 pts experienced 111 infections ≥G2 including also zoster reactivations (4), nodal tuberculosis (1) and Can-

dida-related esophagitis (1). The only feature associated with increased infectious risk was a history of previous infection. Overall, 110 pts discontinued RUX after a median time of 12.5 mos; RUX discontinuation was more frequent in pts ≥75 yr (47.7% *vs* 34.5%, p=0.03). Evolution into acute leukemia (AL) occurred in 9 (8.3%) pts aged ≥75 and in 13 (7.7%) elderly pts. After a median follow-up of 19.5 mos, 65 pts died because of: progression of myelofibrosis (40%), heart disease (10.8%), infections (13.8%), AL (15.4%), hemorrhage/thrombosis (7.7%), transplant (4.6%), other causes. Together with older age, only a BMI<21, corresponding to the first quartile, was significantly associated with worse survival (p=0.02). Since elderly pts had SR/SyR and toxicity rates comparable to those observed in the general population, older age per se should not be a limitation for treating pts with RUX. The assessment of comorbidities, nutrition status and infectious history may identify specific pts fragilities.

Table 1. Patients' characteristics according to age at RUX start.

Characteristics	Age <75 years (n. 168)	Age ≥75 years (n. 109)	*p value*
Male sex, no. (%)	100 (59.5%)	63 (57.8%)	0.78
Primary Myelofibrosis, no (%)	89 (53.0%)	59 (54.1%)	0.85
JAK2 ^{V617F} mutation, no (% on 232 evaluable)	115 (79.9%)	75 (85.2%)	0.30
IPSS intermediate-2/high, no (%)	149 (88.7%)	105 (96.3%)	0.024
Median hemoglobin, g/dl (range)	10.6 (6.8-15.8)	9.9 (5.0-16.7)	0.29
Hemoglobin <10 g/dl	71 (42.3%)	57 (52.3%)	0.10
Transfusion dependence, no (%)	47 (28.0%)	39 (35.8%)	0.17
Median platelet, x10^9/l (range)	289 (52-1632)	211 (33-1887)	<0.001
Platelet >200 x10^9/l	116 (69.0%)	56 (51.4%)	**0.003**
Constitutional symptoms, no (%)	77 (45.8%)	61 (56.0%)	0.053
Palpable spleen, no (%)	159 (94.6%)	108 (99.1%)	0.11
Spleen ≥10 cm, no (%)	111 (66.1%)	72 (66.1%)	0.99
Unfavorable karyotype, no (% on 136 evaluable)	8 (9.2%)	1 (2.0%)	0.11
Median CCI (range)	2 (0 – 11)	3 (0 – 10)	**0.009**
CCI ≥2, no (% on 226 evaluable)	72 (51.4%)	58 (67.4%)	0.018
CCI ≥3, no (% on 226 evaluable)	53 (37.9%)	45 (52.3%)	0.033
Median BMI (range)	23.8 (16.7-33.3)	23.8 (15.6-31.2)	0.21
Grade 3 marrow fibrosis, no (% on 254 ev)	42 (27.3%)	38 (38.0%)	0.07
Time from MF diagnosis to RUX start >2 years	77 (45.8%)	45 (41.3%)	0.46
RUX starting dose			
5 mg BID	15 (8.9%)	23 (21.1%)	**0.004**
10 mg BID	19 (11.3%)	8 (7.3%)	0.28
15 mg BID	39 (23.2%)	29 (26.6%)	0.52
20 mg BID	95 (56.5%)	49 (45.0%)	0.06
RUX 12weeks titrated dose			
5 mg BID	34 (20.6%)	35 (32.4%)	**0.03**
10 mg BID	33 (20.0%)	15 (13.9%)	0.26
15 mg BID	48 (29.1%)	24 (22.2%)	0.26
20 mg BID	50 (30.3%)	34 (31.5%)	0.69

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A PROSPECTIVE OBSERVATIONAL EPIDEMIOLOGIC STUDY OF PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS: THE EXPERIENCE OF LATIUM NETWORK FROM 2011 TO 2015

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Background: Philadelphia Negative Myeloproliferative Neoplasms (Ph-MPNs) are clonal hematopoietic disorders including Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF). To date available epidemiological studies of Ph-MPNs, and in particular prospective evaluations, are rare. In this report we aimed to evaluate in a prospective manner the incidence of Ph-MPN and its variability across five years in the Latium a region with about 6.000.000 residents. In addition, a report of the general features of our population is given. *Methods:* This prospective epidemiologic analysis is based on 1137 consecutive adult patients affected by MPNs (PV=299, ET=561, PMF=214), the remaining 63 pts were diagnosed as MPNu (undeterminated), according to 2008 WHO criteria, from January 2011 to December 2015 in 14 hematological Centers (5 academic and 9 community-based hospitals) in the Latium region. *Results:* The overall incidence rates of PV were $1.0/10^5$ in 2011 and 2012, $1.1/10^5$ in 2013, $0.9/10^5$ in 2014 and 2015. The overall incidence rates of ET were $2.0/10^5$ in 2011, $2.4/10^5$ in 2012, $2.2/10^5$ in 2013, $1.8/10^5$ in 2014 and $1,2/10^5$ in 2015. The overall incidence rates of PMF were $0.7/10^5$ in 2011 and 2012, $1.0/10^5$ in 2013, $0.7/10^5$ in 2014 and $0.5/10^5$ in 2015. The overall incidence rates of MPNu were $0.3/10^5$ in 2011 and 2012, $0.14/10^5$ in 2013, $0.24/10^5$ in 2014 and $0.22/10^5$ in 2015. Baseline features of PV, ET and PMF patients, symptoms at onset, antithrombotic prophylaxis and initial chemotherapies are summarized in Table 1. Thirty-five percent of patients presented at diagnosis other comorbidities requiring specific treatments: the incidence of a previous or concomitant neoplasia was 4%. Among the main thrombotic risk factors, diabetes was present in 11.8%, dyslipidemia in 16.2%, smoke attitude in 13.2%, essential hypertension in 51.7% of total patients. *Conclusions:* In this epidemiological study we confirm, in a prospective way, the overall incidence of Ph-MPN previously reported in other cancer registries and, in particular, the higher incidence rates of ET in comparison to the other Ph-MPN, with a prevalence of male sex in PV and PMF and female sex in ET. Moreover, it is worth noting that the annual incidence was stable during the observational period in all the different types of Ph-MPN.

Table 1. Baseline features according to MPN type, symptoms and treatment of patients in the prospective cohort.

	PV	ET	PMF
Total Number	**299**	**561**	**214**
M/F	165/134	209/352	126/88
Median Age(years)	67.7	66.1	71.1
Median WBC count (x 10^9/l)	9.70	8.60	9.17
Median PLTs count (x 10^9/l)	464	715	441
JAK-2 ^{V617F}pos (%)	93	66	48
CALR pos (%)	0	29	16
Spleen enlargement > 5 cm (%)	5	2	33
Liver enlargement >5 cm (%)	1.3	0.7	4.3
Previous thrombosis (%)	16.6	17	13
Symptoms:			
Pruritus (%)		5.1	
Astenia (%)		5.6	
Headache (%)		3.8	
Vascular (%)		4.6	
Sistemic (Fever, weight loss, sweats) (%)		6.7	
No symptoms(%)		73.9	
Initial Therapy:			
HU alone (%)		65.5	
Anagrelide (%)		2.0	
INF alfa (%)		2.0	
JAK2 Inhibitor (%)		3.0	
No therapy (%)		8.4	
Pipobroman (%)		1.1	
Other (danazole, phlebothomy) (%)		18.0	
Antithrombotic prophylaxys:			
Aspirin (%)		71.4	
Oral Anticoagulants (%)		10	
Other (Clopidogrel, Ticlopidine) (%)		9.2	
No therapy (%)		9.4	

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RUXOLITINIB AND VITAMIN K ANTAGONISTS(VKA) IS SAFE AND EFFECTIVE AND SHOWS A FASTER RECANALIZATION AND AN ANTINFLAMMATORY EFFECT IN PATIENTS WITH PRIMARY MYELOFIBROSIS(PMF) WITH SPLANCHNIC VEIN THROMBOSIS(SVT)

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Aim: Aim of this case series is to analyze if use of ruxolitinib+VKA is safe and effective in treatment of patients withPMF with IPSS INT-2. *Patients and Methods:* This study is a retrospective study. 4 female patients, median age47(R35-55), withPMF INT-2 and with SVT(2portal, 2mesenteric+splenic+portal),median Hb11.5g/dl(R11-12.5), PLT90000/mcl(R70000-100000), WBC10000/mcl(R4000-11000), peripheral blood

blasts 1%(R1-2), 1 patient heterozygous for factor V Leiden received ruxolitinib20mg/day+warfarin. All patients were Jak-2 mutated(GROUP1). In an historical cohort 6patients 2male,4female median age60(R45-65), 3PV,1ET,1PMF, 1 paroxysmal nocturnal hemoglobinuria and with SVT(4portal,2splenic+portal),median Hb12.5g/ dl(R10-13.5), PLT150000/mcl(R110000-200000), WBC8000/ mcl(R6000-10000), peripheral blood blasts 0%(R0-1), 1 patient heterozygous for factor V Leiden, 1 for prothrombin G20210, received Hydroxyurea 1000 mg(R500-1500)/day+warfarin. 3 patients were Jak-2 mutated(GROUP2). In another historical cohort 6patients 4male, 2female median age60(R55-70), 3liver cirrhosis, 3solid cancer and with SVT(5portal, 1splenic+portal), median Hb11.5g/dl(R9-12.5), PLT100000/mcl(R90000-130000), WBC4000/mcl(R2000-9000), peripheral blood blasts 0%,received only warfarin. 1 patient was Jak-2 mutated(GROUP3). *Results:* Patients of GROUP1 showed a complete resolution of SVT in 2 cases and a partial portal recanalization in 2 cases after 3 months, without any thrombosis relapse or progression after 6 months; patients of GROUP2 showed a complete resolution of SVT in 2 cases, a partial portal recanalization in 2 cases and no resolution in 2 cases only after 6 months; patients of GROUP3 showed a partial portal recanalization in 3 cases, no resolution in 2 cases and 1 progression only after 6 months. MedianCRP after0, 3and6 months(mg/l) was respectively inGROUP1 27(R18-33),8(R3-10),6(R3-7),inGROUP2 30(R20-35),18(R12-22),10(R7-15),in GROUP3 38(R28-40),35(R27-42),33(R22-35). MedianCirculating Endothelial Cells/ml inGROUP1 after0, 3and6 months(mg/l) were respectively 1500(R800-5500),800(R600-1800),500(R400-1200). No patient showed side effects treatment related. *Conclusions:* Ruxolitinib and VKA is safe and effective and shows a faster recanalization and an antinflammatory effect in patients with PMF with SVT. These results needs confirmation on a larger cohort of patients.

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COMORBIDITIES AND BODY MASS INDEX IMPACT ON SURVIVAL IN PATIENTS WITH MYELOFIBROSIS TREATED WITH RUXOLITINIB

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Ruxolitinib (RUX) may induce spleen/symptom responses and improve quality of life in myelofibrosis (MF). No data are available on the role of comorbidities and body mass index (BMI) on RUX-treated pts. We evaluated the impact of the Charlson comorbidity index (CCI) and BMI on overall survival (OS) in a cohort of 343 MF pts treated with RUX in 20 Hematology Centers in Italy. Response to RUX was evaluated according to the 2013 IWG-MRT criteria. OS was calculated from the date of RUX start to death or last follow-up. Between June 2011 and Apr 2016 (median follow-up 3.6 years), 343 pts with PMF (51.9%), or post-ET (20.1%)/post-PV MF (28.0%) were treated with RUX. At RUX start, median age was 67.6 years (range 36.5-89.0); IPSS was intermediate (intm)-1 (16.0%), intm-2 (47.5%), high (36.4%). Transfusion dependence and spleen enlargement were present in 23.9% and 97.4% of pts, respectively. TSS was <20 in 131 pts (38.2%); 62 (18.1%) pts had a BMI <21 (corresponding to the lower quartile). CCI was 0 in 105 pts (30.6%), 1 in 74 pts (21.6%), 2 in 58 pts (16.9%) and ≥3 in 106 pts (30.9%). Median RUX exposure was 21.2 months (3-56.2). In multivariable Cox regression analysis, the factors that correlated negatively with OS from the start of RUX were: transfusion dependence (HR: 2.65; p<0.001), CCI ≥3 (HR: 1.67; p=0.031), BMI <21 (HR: 1.74; p=0.039) and IPSS (intm-2=HR 3.19; p=0.057; high risk=HR 6.83; p=0.001). Scoring values were assigned to each factor based on multivariable HR values (transfusion dependency=1.5; CCI≥3=1; BMI <21=1; intm-2=2 and high risk=4) and 3 different groups were identified: group1 (0-2 points, 137 pts), group 2 (3-5 points, 144 pts) and group 3 (5.5-7.5, 62 pts). The OS at 3 yrs was 91.8%, 65.6% and 34.8% for groups 1, 2 and 3, respectively (p<0.001). While 88.7% of high IPSS risk pts clustered in group 3, only 60.5% of pts in group 1 were at intm-2 risk and 48.6% of pts in group 2 were at high risk. The achievement of a spleen response at 6 months (39.2% *vs* 36.4%, p=0.71) was not influenced by a lower BMI. However, pts achieving a spleen response at 6 months significantly increased OS (Figure 1A). Also, a higher CCI did not correlate with lower spleen response at 6 months (44% *vs* 34% of pts with CCI<3, p=0.11). The impact of a higher CCI on survival was only mildly affected by the achievement of a spleen response at 6 months (Figure 1B). CCI and BMI may influence OS in RUX-treated pts. The achievement of a spleen response counterbalanced the negative prognostic effects of a lower BMI.

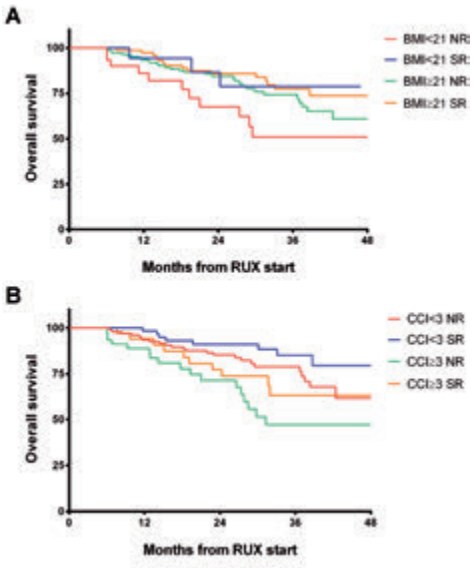

Figure 1.

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ERYTHROPOIETIN STIMULATING AGENTS GREATLY IMPROVE ANEMIA IN PATIENTS WITH MYELOFIBROSIS TREATED WITH RUXOLITINIB

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Anemia is common in patients with myelofibrosis (MF) and it is one of the main cause of symptoms in this setting. Erythropoiesis stimulating agents (ESA) have been used in MF with anemia response rate ranging between 23 and 60% in different reports. Ruxolitinib is highly effective in reducing spleen size and controlling the symptoms of MF, thus resulting in a marked improvement in the patients' quality of life and possibly a prolonged survival. However, one of ruxolitinib main side effects is anemia, which occurs in 40% of the patients and can be a limiting factor for treatment tolerability and thus compliance and optimal dosage, mostly in the first weeks of treatment. We retrospectively

evaluated 42 patients who received concomitant therapy with ruxolitinib and ESA to evaluate the efficacy and safety of this combination therapy. ESA (epoetin alpha/beta/zeta or darbepoetin) were given off-label after obtaining patient written consent and local pharmacy approval. Erythroid response was defined according to IWG-MRT criteria. Median age at ESA start was 69 years (range 48-81). 75.5% of patients were at intermediate 2 and 24.5% at high risk according to DIPSS. Thirty-five patients received ESA after ruxolitinib start: 9 were already RBC transfusion dependent before commencing ruxolitinib while 14 patients required red blood cell (RBC) transfusions only after treatment start. Overall ruxolitinib treatment worsened anemia leading to RBC transfusion requirement in 86% of previously transfusion independent patients. Overall response rate to ESA was 90%, with 71% of erythroid response and 19% of partial response. Median time to response was 4 months and 65% of patients were still responding at 4 years. Erythroid response had also a positive impact on survival. Sixty-six% of patients responded to ruxolitinib in terms of spleen size and a spleen increase during ESA treatment was observed in 2 patients only. No thrombotic events and no toxicity were reported over treatment with ESA. In conclusion ESA were effective in improving anemia in MF treated with ruxolitinib. We observed a high response rate in this patients series without significant toxicities. In particular no thrombotic event and no impact on response to ruxolitinib were reported. This results may suggest a synergistic activity of ESA and ruxolitinib, maybe for its activity in reducing splenomegaly and inflammatory symptoms. This is an important finding that should be confirmed prospectively.

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CLINICAL BEHAVIOUR OF ESSENTIAL THROMBOCYTHEMIA IN PAEDIATRIC AGE

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Patients affected by Essential Thrombocythemia (ET) are prone to thrombotic and haemorrhagic complications. ET is extremely rare in paediatric age and little is known about the clinical lapse of this disease in children. Moreover, while in the past the diagnosis of ET in children was based only on clinical features, now WHO criteria that consider mutational status and histological features are available. We therefore found interesting to report the clinical events observed in 20 children (14 males and 6 females, mean age at diagnosis 10 y, range 1-16; median follow up 4.6y, range 1-11) strictly diagnosed in agreement with WHO 2016 criteria. The children's median platelet count was $1251x10^9$/L (range 528-1824), WBC $8.2x10^9$/L (range 5.5-11.8), haemoglobin 132g/L (range 113-141). Six patients carried JAK2V617F, 2 CALR and 1 MPL mutations, the remaining 11 children were not mutated (triple negative: 3NEG). In all patients, bone marrow histology was compatible with ET. Splenomegaly was documented in 13 patients (65%) and abdominal pain in 3 boys and in 1 female (20%). Eight children suffered for microvascular disturbances (2 JAK2, 1 CALR, 1 MPL and 4 3NEG), 2 (1 JAK2 and 1 CALR) had minor bleedings and 3 (2 JAK2V617F and 1 3NEG) major thrombosis (2 transient ischemic attacks [TIA], 1 Budd-Chiari syndrome). Taking together, only 3 children did not have any clinical sign. We failed to recognize any relation between clinical event and sex, age, mutational status, or platelet counts. The presence of splenomegaly appears more common in children than in adults. Abdominal pain is typical of children with ET and its cause is at present unknown. In the absence of evidence for risk stratification that should be useful to guide therapeutic decision, our data show that major complications (*i.e.* thrombotic events) can occur in this rare setting of patients. In agreement with what is known in adult settings, low dose aspirin has to be used in patients with TIA and long lasting anticoagulation is indicated in Budd Chiari syndrome. Cytoreductive drugs has to be considered in patients with thrombosis in spite of their possible adverse effects considering the life-treating risk of a new event. In contrast, in other patients, wait and watch is probably the best policy.

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ADVANCED CHRONIC MYELOMONOCYTIC LEUKEMIA IN ELDERLY AND FRAIL PATIENTS MANAGED BY AZACITIDINE IN A REAL LIFE SCENARIO

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Significant clinical benefits by azacitidine (AZA) have been reported in chronic myelomonocytic leukemia (CMML) patients with an overall response rate (ORR) ranging from 39% to 60% and a median survival (OS) from 12 to 37 months. We report the outcome of 22 unselected CMML patients (median age 75 years) treated with AZA, to evaluate its efficacy in a "real life" scenario. Of the 22 cases, 20 (91%) had CMML-2, 1 CMML-1 with severe and symptomatic cytopenias and 1 CMML-related AML with <30% BM blasts. Twelve patients (55%) had MD-CMML, whereas 10 (45%) MP-CMML who received AZA as salvage treatment after failure of disease control by hydroxyurea. Thirteen patients (59%) were transfusion-dependent. Abnormal karyotype was observed in 4 patients. According to CPSS, 2 (9%), 12 (55%), 7 (32%) and 1 (4%) patients belonged to the low, int-1, int-2 and high risk group, respectively. AZA was started after a median of 3 months from CMML diagnosis (range 1–18). The median number of AZA courses was 18 (2-39) without remarkable side effects. Of the 22 patients, 7 (32%) achieved CR and 9 (40%) PR with an ORR of 72%; 6 patients (28%) presented a primary failure to AZA (3 SD and 3 PD, respectively). After a median follow-up of 25 months (range 5-51), median OS was 17,3 months from the start of AZA with 5 patients still alive (1 after unrelated SCT, 1 in CR, 2 in PR and 1 with SD) after 36, 24, 18, 20 and 32 months respectively. Patients who responded to AZA showed a significant better survival comparing to non-responders (27 *vs* 13 months, respectively, p=0,001); no difference was observed by stratifying for sex, CPSS and the MD *vs* MP CMML. Of the 17 (77%) patients who deceased, 8 (37%) died of complications unrelated to the disease or the hypomethylating treatment, whereas 9 (40%) after a median of 3 (1-12) months from AML progression, developed after a previous response to AZA in 6 cases. The AML transformation-free probability of responding patients was significantly longer in responders than non-responding patients (41 *vs* 10 months respectively, p=0, 02), while CPSS 1-2 (20,7 months) *vs* CPSS 3-4 (14,8 months) showed no significant differences. Although in a limited number of patients, our results are encouraging indicating AZA as a suitable treatment of elderly CMML in a real-life scenario.Future studies to identify predictive molecular signatures for sensitivity to azacitidine reliable also in the daily clinical practice and the development of new treatment approaches based on combination of epigenetic agents with novel compounds are highly awaited in this difficult setting.

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INCIDENCE OF THROMBOSIS IN PRIMARY AND SECONDARY MYELOFIBROSIS: A RETROSPECTIVE MONOCENTER STUDY

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Primary myelofibrosis (PMF) is a myeloproliferative neoplasm with poor prognosis, characterized by bone marrow failure, splenomegaly, constitutional symptoms, leukemic progression and shortened survival. Secondary myelofibrosis (SMF) evolved from polycythemia vera (PV) or essential thrombocythemia (ET) show similar features. The incidence of thrombotic complications has been rarely investigated, and seems not negligible either in PMF and in SMF. We retrospectively investigated 59 patients with PMF (M/F 34/25, median age at diagnosis 61 yrs, range 29-86) and 52 patients with SMF, in 19 cases after PV and 33 after ET (M/F 17/35, median age at MF evolution 61 yrs, range 16-88); total observation years were 255 for PMF and 204 for SMF. We recorded 14 first major thromboses of arterial (n=5) or venous vessels (n=9) in PMF patients (23.7%); in 9 cases (15.2%) thrombosis was the heralding event leading to diagnosis of PMF. Three patients had recurrences, two arterial and one venous; one of them had a second arterial recurrent thrombosis. Therefore, after the exclusion of the heralding events, the rate of

overall incident thrombosis during the follow up was 3.5 per 100 pt-yrs (95%CI 1.61-6.70). During the follow-up the incidence rate per 100 pt-yrs of first and recurrent thrombosis was 1.96 (95%CI 0.63-4.57) and 6.25 (95%CI 1.8-18.26), respectively. PMF patients with (n=14) or without thrombosis (n=45) did not differ in the rate of male sex, age >60 years, history of remote thrombosis, thrombophilia, JAK2 V617F mutation, splenomegaly, Hb <10 g/dL, WBC >25,000/mmc, platelets <100,000/mmc, constitutional symptoms. Antiplatelet treatment did not prevent thrombosis (odds ratio, OR, 0.55, 95%CI 0.16-1.85), whereas cytoreduction with hydroxyurea reduced the thrombotic risk by 78% (OR 0.22, 95%CI 0.05-0.81). Six patients with SMF had thrombosis since the time of evolution, one arterial and 5 venous (11.5%); 4 of them had previously suffered from thrombosis before evolution to MF. Therefore the incidence rate of first thrombosis after diagnosis of SMF per 100 pt-years was 0.98 (95%CI 0.11-3-54), without significant difference in comparison with PMF (p=0.39). In conclusion the rate of first thrombosis either in PMF and SMF is comprised between 1 and 2 per 100 pt-years; the recurrence rate is comparable to that observed in patients with PV or ET. Significant prevention of thrombosis can be achieved by cytoreduction, whereas antiplatelet treatment seems ineffective.

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THROMBOTIC RISK ASSOCIATED WITH JAK2 V617F MUTATION AND INHERITED OR ACQUIRED THROMBOPHILIA IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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In patients with Philadelphia-negative myeloproliferative neoplasms (MPN) JAK2V617F mutation is a well established risk factor for thrombosis; the interaction with the presence of inherited and/or acquired thrombophilia has been scarcely investigated. We retrospectively investigated 704 patients with MPN and a complete investigation for thrombophilia from an overall cohort of 902 patients: 231 with polycythemia vera (PV) (M/F 123/108, median age at diagnosis 57 yrs, range 21-92), 433 with essential thrombocythemia (ET) (M/F 140/293, median age at diagnosis 52.5 yrs, range 10-92), and 40 patients with primary myelofibrosis (PMF) (M/F 20/20, median age at diagnosis 60 yrs, range 29-82). The JAK2V617F mutation was present in 209 PV pts. (90.4%), 265 ET pts. (61.2%), and in 26 PMF pts. (65%). All patients were investigated for the presence of deficiency of natural anticoagulants (antithrombin, proteins C and S), factor V Leiden, prothrombin G20210A, hyperhomocysteinemia, lupus anticoagulant, anticardiolipin, and antibeta2glycoprotein I antibodies. Overall, a thrombophilic trait was detected in 206 pts. (29.2%). A thrombotic event occurred in 228 pts. of the investigated cohort (32.3%); among them, JAK2V617F or thrombophilia was present in 191 (83.7%) and 83 (36.4%) individuals, respectively; combination of both was present in 73 of them (32%). The odds ratio for thrombosis was 2.79 (95%CI 1.87-4.16, p<0.0001) for JAK2 V617F, and 1.64 (95%CI 1.17-2.30, p=0.0046) for thrombophilia. The combination of both JAK2V617F and thrombophilia was associated with a risk for thrombosis 3.99-fold (95%CI 2.38-6.68, p<0.0001) in comparison with the JAK2V617F-negative and thrombophilia-negative patients. The magnitude of risk associated with the combination of JAK2V617F and thrombophilia was 5.46 (95%CI 2.76-10.82, p<0.0001) in patients <60 years and 2.57 (95%CI 1.15-5.70, p=0.023) in patients >60 years. Single carriership of JAK2V17F or thrombophilia was still significantly associated with thrombosis in the patients <60 years, with a magnitude of risk similar to that found in the whole cohort (data not shown), but not in the patients >60 years. In conclusion either JAK2V617F mutation and thrombophilia are associated with a significant risk for thrombosis in MPN, mainly in the younger patients, and the combination of both produces an additive risk. The risk associated with double carriership of JAK2V617F and thrombophilia remains significant also in the older patients.

Myeloproliferative Syndromes 2

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SUSTAINED ERYTHROPOIETIC RESPONSE IN A PATIENT WITH MYELOFIBROSIS RECEIVING CONCOMITANT RUXOLITINIB AND DEFERASIROX

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Background: Myelofibrosis (MF) is a myeloproliferative neoplasm frequently complicated by transfusion dependent anemia. Both anemia and transfusion-dependence are associated with a poor outcome, at least in part because of toxic effects of iron overload (IOL). Recent studies have shown that deferasirox favourably impacts on the clinical outcome of patients. However, information dealing with hematologic responses to deferasirox in transfusion-dependent patients with MF are limited. *Case report:* We report here a 54 -year-old woman with a long-term diagnosis (*i.e.*, 20 years) of essential thrombocytemia (TE) treated with hydroxyurea. In July 2013 patient evolved to secondary MF (i.e, BM fibrosis grade II) which was scored according to IPSS score as intermediate- 2 due to the presence of constitutional symptoms and transfusion-dependent anemia. A 3-month treatment with Ruxolitinib (*i.e.*, 20 mg bid) led to a consistent shrinkage of splenomegaly (*i.e.*, from 20 to 12 cm) and to the disappearance of constitutional symptoms while persistent transfusion-dependent anemia produced meaningful increase of serum ferritine (*i.e.*, 3827 mg/dL). Due to considerable IOL, patient was commenced on oral deferasirox 15 mg/kg daily. Persistent grade 2 diarrhea limited the adherence to the therapy with deferasirox which was finally stopped after 6 months. In September 2015, deferasirox therapy was restarted at a lower dose (*i.e.*, 7,5 mg/Kg/ day) and gradually increased up to 15 mg/Kg/ day. Gastrointestinal side effects were negligible whereas transfusion dependence dropped to one unit of packed red blood cells per month. Noteworthy, the patients in a couple of months reached and maintained haemoglobin levels that ranged between 9 and 10 g/L in absence of trasfusional support. The sustained erythropoietic response, maintained for about 9 months, was associated with a steady ferritin serum level lower than 1000mg/dl. *Conclusions:* In conclusion, this case report highlights that the positive erythropoietic effects of deferasirox, previously reported in myelodysplatic syndromes, can be extended to patients with MF.

P115

RISK OF PROGRESSION TO MYELOFIBROSIS IN ESSENTIAL THROMBOCYTHEMIA: IMPACT OF CALR MUTATIONS

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Background: In Essential Thrombocythemia (ET) 85% of patients (pts) harbors one of three driver mutations with mutational frequencies of approximately 58%, 23% and 4% for JAK2, calreticulin (CALR) and MPL, respectively; almost 15% of pts is wild-type for all three mutations and is operationally called "triple-negative" (3NEG). Several studies identified typical clinical and hematological features according to mutational genotype. CALR mutations seem to define a clinical picture of an indolent disease with lower risk of thrombosis. However, controversial data have been reported regarding long-term survival and risk of myelofibrosis (MF) progression in CALR mutated ET. The aim of this study is to examine the impact of CALR mutations on overall survival (OS) and myelofibrosis-free survival (MFS). *Methods:* We collected 327 pts affected by ET referred in our Hematology Division from 1995 to 2016. All pts were annotated for driver mutations, in particular 62 pts (18.9%) presented CALR mutations (64.3% type-1 DEL, 35.7% type-2 INS), whereas JAK2, MPL and 3NEG were found in 205(62.7%), 11(3.4%) and 49(15%) pts, respectively. MF evolution was considered for MFS, where death prior MF was set as right censored. Survival probability was estimated by Kaplan Meier estimator with consideration of competing risks; Log Rank test was used for comparison. Impact of co-

variates on hazard of failure was assessed by Cox semi-parametric regression model. MPL mutated pts were excluded due to the small sample size. *Results:* We observed a higher number of MF evolution in CALR group with respect to JAK2 and 3NEG pts (22.6% *vs* 6.83% *vs* 8.2%, respectively). In univariate analysis (Figure 1), MFS showed a separation across driver mutations (p≈0.05). In the multivariate Cox regression model, including sex and age, CALR mutation significantly increased the hazard of MF progression with respect to the other two groups (p=0.014; HR=2.75) together with prior thrombosis (p=0.02; HR=2.86) and extreme platelet counts at diagnosis (p=0.05; HR=2.11). Particularly, the presence of type-1 DEL CALR mutation was associated with an increased risk of MF progression (p=0.05), with respect to type-2 INS CALR one. Finally, there was not significant difference in OS among the other driver mutational categories. *Conclusions:* CALR mutated pts might be at higher risk of progression to MF, whose onset has to be considered however as a late event in the natural history of the disease, not influencing the OS.

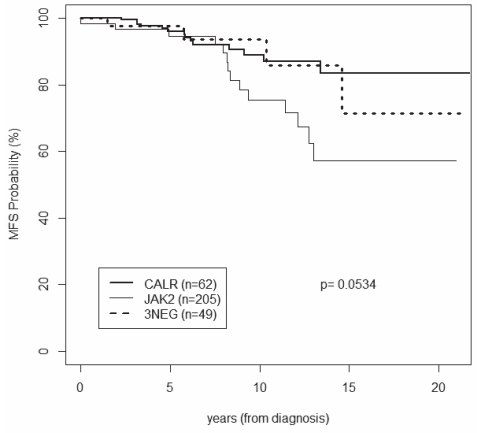

Figure 1. Univariate analysis of MFS probability according to driver mutations (p≈0.05).

P116

AN UNUSUAL TYPE OF MYELOID SARCOMA LOCALIZATION FOLLOWING MYELOFIBROSIS

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Myeloid sarcoma (MS) is defined as "a tumor mass consisting of myeloid blasts with or without maturation occurring at an anatomic site other than the bone marrow". It can present as a *de novo* malignancy (without leukemic presentation) during the course of AML, MDS or MPN, but also as a relapse of a previous AML. MS most commonly involve the lymph nodes, skin, soft tissues and testis, with a multifocal presentation in less than 10% of cases. Here we report an unusual case of MS presenting with multiple skin, subcutaneous and muscular lesions in a patient affected by myelofibrosis (MF) which was diagnosed about 16 years before. A 53-years-old man was diagnosed with pre-fibrotic MF in 2000. Subsequent molecular evaluations demonstrated the absence of JAK2V617F mutation. Eleven years later, because of appearance of severe anemia a bone marrow biopsy was performed and showed an increase in bone marrow fibrosis (MF-2). Further molecular tests showed mutation of CALR gene (ins5-bp). In September 2016 the patient presented with asymptomatic subcutaneous nodules on the chest wall, neck and left arm (2cm). A FDG-PET detected multiple metabolically active lesions on soft tissues (SUVmax 4.5) on right and left shoulder, chest wall, back, epigastric region, and legs. A subcutaneous excisional biopsy revealed the presence of a granulocytic sarcoma (the majority of proliferating cells expressed CD34, CD43, CD117(+/), CD45/LCA(+/-) antigens, while were negative for CD20, CD3, CD30, CD68/kp1, CD68R antigens and for MPO). Immunohistochemical positivity of NPM1 with nuclear dislocation was also present, whereas FLT-3 mutations were not found. Subsequent bone marrow biopsy showed leukemic transformation of MF, with a normal male karyotype. The patient underwent "3+7" induction chemotherapy, followed by consolidation with 3 courses of intermediate dose Ara-C, obtaining complete bone marrow remission, clinical disappearance of subcutaneous nodules, but persistence of FDG-PET pathological uptake on both arms. In conclusion MS and in particular MS following MF is a rare entity. Indeed, in the two largest patients series reported so far, only few patients had a diagnosis of MS concurrent or after MF. Furthermore, muscles involvement is reported as anecdotal. Since its pathogenesis and genomic landscape are not well established, the prognosis remain dismal, even in the novel agent era. Therefore, each case description is fundamental to enhance our knowledge about this disease.

P117

EFFICACY AND SAFETY OF DEFERASIROX IN THE MANAGEMENT OF IRON OVERLOAD IN MYELOFIBROSIS ALSO IN PATIENTS TREATED WITH RUXOLITINIB

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Background: Deferasirox (DFX) is the principal option currently available for iron-chelation therapy (ICT), principally in the management of myelodysplastic syndromes, while in myelofibrosis (MF) the expertise is limited. In particular few data are available in the setting of MF patients (pts) treated with JAK2 Inhibitors. *Methods:* We analyzed our experience in 18 pts with primary MF (n=13) or post-Polycythemia MF (n=2) and post-Essential Thrombocythemia MF (n=3), in chronic phase, with transfusion-dependent anemia, treated with DFX from September 2010, in order to evaluate the efficacy and safety profile of this approach in MF pts, including those treated with Ruxolitinib. *Results:* DFX was started after a median interval from diagnosis of 31.2 months, with median ferritin values of 1734 ng/mL (IR:1264-3198). The median dose tolerated of DFX was 750 mg/day (10mg/kg/day).

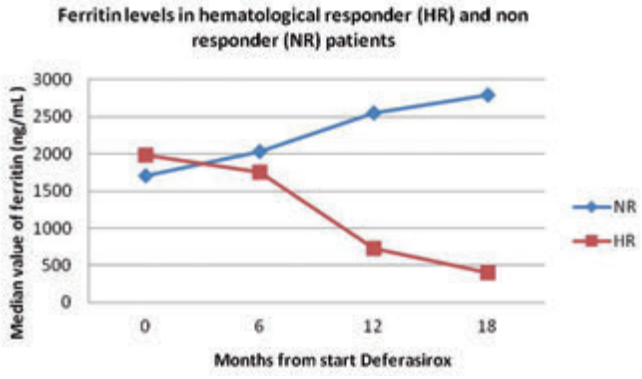

Figure 1.

Extra-hematologic toxicity was reported in 7/18 pts (38.8%), principally consisting in transient renal or hepatic impairment, but only 4 pts discontinued definitively treatment due to toxicity. After a median treatment period of 12.3 months (IR: 6-49.1), according to IWG 2006 criteria, an erythroid response (HR) with DFX were observed in 8/18 pts (44.4%), 4 of them (22.2%) obtained transfusion independence (TI). The median time to best HR was 3 months. Focusing on pts treated with Ruxolitinib (n=7), regarding the hematologic improvement, 3/7 pts (42.8%) showed an HR with reduction of 50% of transfusional support, but none of them obtained TI. Only 2 pts (28.5%) presented extra-hematologic toxicity (renal dysfunction), conditioning discontinuation of the drug. After a median follow-up of 50 months, 10 pts (55.5%) died, 4 of them for leukemic evolution (MF-BP). In the setting of Ruxolitinib pts, only 1 patient died (due to an infective complication). Overall, pts with HR receiving DFX showed a progressive reduction of ferritin levels at 6, 12 and 18 months respect to baseline (Figure 1), and we observed a trend for a better overall survival from DFX initiation (31.8 *vs* 22.2 months) and a lower incidence of MF-BP (4 pts *vs* 0) in HR

respect to non responder pts. *Conclusions:* Treatment with DFX is feasible and effective in MF with ICT, including the subgroup of pts treated with Ruxolitinib. Moreover, in the MF setting, haematological improvement can occur in a significant proportion of pts and the obtainment of HR to DFX seems to be associated with better survival and lower incidence of MF-BP.

P118

ADHERENCE TO TREATMENT IN PATIENTS WITH MYELOFIBROSIS: PRELIMINARY VALIDATION OF A QUESTIONNAIRE

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La mielofibrosi e' una malattia mieloproliferativa Philadelphia negativa in cui non è individuabile nessuna mutazione driver nel 5-10% dei pazienti (Tripli negativi).Le attuali linee guida indicano che i pazienti con mielofibrosi possono essere sottoposti a terapia citoriduttiva e Ruxolitinib,nelle fasi piu' avanzate.Scopo del nostro studio e' stato quello di valutare l'aderenza alla terapia medica (idrossiurea, ruxolitinib) dei pazienti con mielofibrosi afferenti al nostro centro. E'stato pertanto ideato un questionario di facile somministrazione ,comprendente 12 quesiti specifici(score 0-1- per ciascun quesito) e validato in maniera preliminare presso la nostra UO. Il questionario prevede il calcolo di un punteggio per cui vengono considerati aderenti al trattamento in corso i pazienti che raggiungono un punteggio uguale o superiore a 9, mentre un punteggio <9 viene considerato indicativo di scarsa aderenza alla terapia.Il questionario è stato somministrato a 115 pazienti con mielofibrosi in terapia con idrossiurea (n=90) o con ruxolitinib (n=25). Al fine di valutare una eventuale correlazione con l'aderenza alla terapia e' stata valutata l' eta' il sesso, il tipo di terapia, i numeri di accesso presso l'ospedale nel corso dell'ultimo anno ed il numero di farmaci che venivano assunti contemporaneamente per comorbidità.I pazienti (58 F, 57 M) avevano al momento dell'indagine una eta' mediana di 71 anni (35-91).E' stata registrata una scarsa aderenza al trattamento (score <9) in 31 pazienti su 115 (26,95%).Nei pazienti sottoposti a terapia con ruxolitinib, l'aderenza alla terapia appare nettamente superiore al gruppo trattato con oncocarbide (rispettivamente 92% e 67,78% ,p=0.01).I pazienti con un numero di accessi in ospedale superiore a quattro negli ultimi 12 mesi mostrano un adesione alla terapia superiore a quella dei pazienti con numero di accessi uguale o minore a 3 accessi. I pazienti in terapia con ruxolitinib, per la particolare modalita' di somministrazione del farmaco, hanno un numero di accessi in ospedale nettamente superiore. L'eta' ,il sesso e ,curiosamente, il numero di farmaci assunti non sono risultati correlati in maniera significativa con l'aderenza alla terapia.In conclusione nei pazienti con mielofibrosi l'aderenza alla terapia rappresenta ancora un problematica aperta.

P119

ANAGRELIDE (ANA) IN ESSENTIAL THROMBOCTYTHAEMIA (ET): PRELIMINARY DATA OF A 25-YEAR EXPERIENCE OF THE "PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS (MPN) LATIUM GROUP"

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Background: The main challenges for the management of Essential Thrombocythemia (ET) are the occurrence of thrombotic/bleeding events, either at diagnosis or during follow-up (FU). The proper drug should prevent thrombotic/bleeding complications, control systemic symptoms, allow management of situations at risk, not to induce transformation into MDS-AL-MF, not to predispose to development of secondary malignancies. ANA is effective to reduce elevated platelet counts in patients (pts) affected by ET and related MPN. *Aims:* To describe the "real life use" of ANA in the last 25 years in ET pts from the Lazio region. *Patients and Methods:* This is an observational, retrospective, multicenter study. ET pts, belonging to "Ph-negative MPN Latium Group" Centers, diagnosed from 1981 to 2012, were recruited. An "ad hoc" CRF was sent to all participant Centers to collect data. *Results:* 150 pts (F101, M49) were enrolled. They started ANA between July 1989 and January 2014 and represented 16% of 938 ET pts followed by the "Latium Group" in need of treatment in the same period. Median age at diagnosis: 42.7y (20.9-87.7); median age at ANA start: 45.9y (22.7-87.2); median FU from diagnosis to the last available control: 12.5y (0.6-33.7). 52/126 pts (41.3%) resulted JAK2V617F positive and 74 (58.7%) negative. ANA was used as first line therapy in 52 pts (34.7%), as second line in 78 (52%) and as third line in 20 (13.3%). Globally, 123/144 evaluable pts (85.4%) responded to ANA in monotherapy: 55 (38.2%) achieved a platelet count <400x109/L and 68 (47.2%) >400 <600x10^9/L; 21 (14.6%) did not obtain a response. During FU, 112/117 (95.7%) evaluable responder pts, maintained the response (platelet count <600x10^9/L) for more than 50% of the treatment period. In responder pts, median daily ANA dose at response was 1.5mg (0.5-5), and during FU, 1.5 mg (0.35-3.5). In 14/148 (9.5%) pts, 10 arterial and 4 venous thrombotic events occurred during FU. In 51/143 pts, bleeding events occurred: 42 minor (29.4%) and 9 major (6.3%). 16/150 (10.7%) pts developed a secondary MF, and 3 (2%) an AL; 12/150 (8%) developed a secondary malignancy. *Conclusions:* ANA was given mostly as second/third therapy line. It was efficacious in reducing platelet count in the majority of pts (85.4%). We observed 9.5% of thrombotic events and 6.3% of major bleedings during FU. As expected, 10.7% of pts developed MF and 2% AL.

P120

DEFERASIROX IN THE TREATMENT OF IRON OVERLOAD DURING MYELOPROLIFERATIVE NEOPLASMS

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At present, very few data are available on the treatment with deferasirox (DFX) in patients with Ph- Myeloproliferative Neoplasms (MPN). To address this issue, we report on 41 patients (M/F 31/10) with MPN and iron overload enrolled in the database of our regional cooperative group, who received a treatment with DFX. According to IPSS classification, 8 patients (19.5%) resulted low/intermediate-1 risk, 14 (34.1%) intermediate-2 risk and 19 (46.4%) high-risk. The main features of the patients at diagnosis and at baseline of DFX treatment are reported in the Table 1.Treatment with DFX was started after a median interval from diagnosis of 13.3 months [interquartile range (IR) 7.3–41.1] and from start of transfusion dependence of 11.5 months (IR 5.8–20.2), with a median of 27 packed red cells units received (IR 18–37). The starting DFX dose was 20mg/Kg in 16 patients (39.1%), 15mg/Kg in 20 patients (48.8%) and 10mg/Kg in 5 patient (12.1%). Extra-hematological toxicity of all WHO grades was reported in 20/41 patients (48.8%) and consisted of gastro-intestinal symptoms in 7 patients, transient renal impairment in 10 patients and skin reactions in 3 patients: however, only 3 patients (7.3%) needed a permanent discontinuation for toxicity. Thirty-nine out 41 patients were evaluable for response (>6 months of treatment). As to chelation efficacy, after a median treatment period of 15.4 months (IR 8.1–22.3), 4 patients achieved ferritin levels <500ng/ml, 10 patients ferritin levels <1,000ng/ml and 2 patients presented a reduction >50% of basal ferritin but with levels >1,000 ng/ml, with a global response rate of 16 out 39 patients (41.0%): among the

remaining 23 patients, 2 discontinued for early toxicity, 20 did not have any ferritin reduction and 1 had an early unrelated death (<6 months of treatment). As to hematological improvement, 7/39 patients (17.9%) showed an unexpected and persistent rise of Hb levels >1.5g/dl, with disappearance of transfusional requirement in 5 cases. The median overall survival (OS) of the whole cohort from DFX initiation was 20.7 months (95% CI 16.0–25.3): the median OS from DFX initiation in patients with chelation response was 46.9 months (95% CI 10.7–83.0) compared to 14.0 months (95% CI 5.6–22.3) in patients without chelation response (p=0.002). Treatment with DFX is feasible and effective in MPN with iron overload. Moreover, patients achieving chelation response had a longer OS.

Table 1. Main patient features at diagnosis and at baseline of DFX treatment.

	DIAGNOSIS	BASELINE
Median age, years [interquartile range (IR)]	70.2 (64.1 – 74.7)	71.5 (67.4 – 76.2)
Median Hb, g/dl (IR)	8.4 (7.3 – 9.7)	7.9 (7.2 – 8.2)
Median ferritin, ng/ml (IR)	504 (171 – 1000)	1502 (1188 – 2255)
Median creatinine level, mg/ml (IR)	NR	1.0 (0.7 – 1.1)

P121

GENE PROFILING OF ENDOTHELIAL PROGENITOR CELLS IN POLYCYTHEMIA VERA PATIENTS REVEALS DISEASE-RELATED SIGNATURES

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Close interactions between hematopoietic and endothelial cells underlie a wide range of biologic processes, including hematopoietic homeostasis and vascular thrombosis. Investigating biological signatures of endothelial progenitor cells in Ph-negative myeloproliferative neoplasms may complement the growing information gained in hematopoietic cells. Endothelial colony forming cells (ECFCs) are circulating CD34+ cells lacking hematopoietic markers; they are able to generate *in vivo* and *in vitro* mature endothelial cells. In order to isolate ECFCs, we cultured mononuclear cells of 21 phlebotomy products collected from 20 patients with JAK2 V617F-positive Polycythemia Vera (PV) and from 55 blood donors as controls. ECFCs were subjected to gene profiling (Human endothelial cell biology PCR array PAHS-015Z, Quiagen, Milan, Italy).

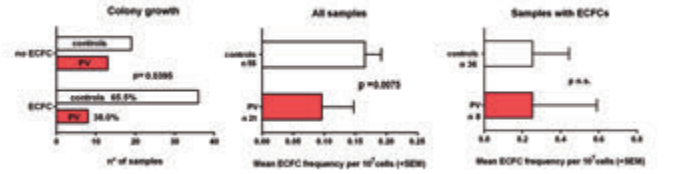

Figure 1.

Data analysis was performed using the RT2 Profiler PCR array data analysis template v3.0 (SABiosciences). Relative changes in gene expression were calculated using Ct (cycle threshold) method. In addition, PV ECFCs were examined for the expression of the JAK2V617F mutation. ECFCs were obtained from 7 out of 20 PV patients (8 out of 21 PV samples) and 36 out of 55 controls (p=0.039). No JAK2V617F mutated colonies were found. Among the 84 genes included in the array, 13 genes were significantly over-expressed (12 genes) or under-expressed (1 gene) in PV cells, independently from the ongoing antithrombotic and cytoreductive therapies (data not shown). Overexpressed genes included matrix-metalloproteinases (MMP: the average fold increase were 23.6 for MMP2, range 7.2-39.6; 20.1 for MMP1, range 2,1-59-2; 3.9 for MMP9, range 2.1-4.8), placental growth-factor (11.3 average fold increase, range 2.0-26.0), plasminogen activator inhibitor-1 (6.7 average fold increase, range 4.8-9.0), heme-oxygenase-1(5.7 average fold increase, range 3.2-14.2). To a lesser extent, protein C receptor, collagen type XVIII alpha-1chain, integrin alpha 5 subunit and BCL-x genes were also up-regulated (all > 2-fold increase in comparison with controls). In addition, PV ECFCs expressed significantly lower levels of tissue-factor-pathway inhibitor gene (7.0 average fold decrease, range 1.5-9.3). These findings highlight possible dysfunctions of JAK2 wild type endothelial progenitor cells, potentially influencing cell interactions within the hematopoietic niches or possibly contributing to the thrombotic phenotype of the disease.

P122

IN JAK2V617F POSITIVE MYELOPROLIFERATIVE NEOPLASMS, ALLELE BURDEN CORRELATES WITH THROMBO-HEMORRHAGIC RISK

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Background: The patients affected by Myeloproliferative Neoplasms (MPN) commonly suffer for thrombotic and hemorrhagic complications. JAK2V617F mutation, which is present in about 95% of patients with Polycythemia Vera (PV), and in more than half of those with Essential Thrombocythemia (ET) or Primary Myelofibrosis, (PMF), has been associated to a higher risk of cardiovascular events. However, the correlation between allele burden (AB) and thrombotic risk is controversial and no data are available about hemorrhages and JAK2 AB. *Methods:* We evaluated 253 consecutive MPN (121 ET, 124 PV and 8 PMF) patients with a median follow-up of 8.8 years (0.1 – 37.3 y) in whom the JAK2 AB was available. Patients were stratified accordingly to their JAK2 AB, into four quartiles (1st <25%, 2nd 26-50%, 3rd 51-75% and 4th >75%). Complete medical history and anti-thrombotic drugs use were recorded. *Results:* In 63 patients (24.9%) cardiovascular events occurred at diagnosis and in 55 (21.7%) during follow-up. Prevalence of thrombosis during follow up was lower in 1st compared both to 3rd (p=0.041) and to 4th (p<0.001) quartiles. Thrombosis-free survival was significantly poorer in 4th quartile compared to 1st one (p=0.003). Three patients (1.2%) bleed at diagnosis, while 27 (11.8%) suffered for hemorrhages during follow-up (10 major and 17 minor). Prevalence of hemorrhages was higher in 4th compared to both 2nd (p=0.003) and 1st (p<0.001) quartiles. Hemorrhages-free survival is lower in 4th quartile compared to 2nd (p= 0.004) and 1st (p<0.001). No statistically significant difference has been demonstrated in the use of anti-thrombotic drugs among quartiles. *Conclusions:* So far, the correlation between AB and thrombo-hemorrhagic risk in MPN is uncertain. In our cohort, we found a significantly higher incidence of both thrombotic and bleeding manifestations during follow-up in patients with higher AB. Our data suggest that MPN patients with JAK2 AB higher than 75% have to be considered as high risk patients, being prone to develop thrombo-hemorrhagic complications during the disease course.

P123

RUXOLITINIB REDUCES SPLENOMEGALY IN PATIENTS WITH MYELOFIBROSIS AND PORTAL VEIN THROMBOSIS: A SINGLE CENTRE EXPERIENCE

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Background: Portal vein thrombosis (PVT) frequently occurs in Myeloproliferative Neoplasms (MPN). It causes portal hypertension that highly contributes to splenomegaly in these patients. Ruxolitinib (RUXO) is a JAK1/2 inhibitor approved for the treatment of splenomegaly or constitutional symptoms associated with myelofibrosis (MF). *Aim:* We report our experience in 3 patients with PVT and secondary MF, diagnosed and followed in our department, treated with RUXO. MM & PATS : Patient 1, a 55 years old man with a diagnosis of PV going up 15 y, suffered for PVT with splenic infarction 3 y after diagnosed. He received RUXO for 20 weeks at 15 BID with the aim of reducing spleen size before allogenic hematopoietic stem cell transplantation (HSCT). Patient 2, a 56 years old female, diagnosed with ET at the age of 30. 3 y later she developed PVT. After 16.5 y she evolved in MF and RUXO was used between 2012 and 2015 when she underwent HSCT. In Patient 3, a 63 years old female, PV arose with PVT at the age of 51. She evolved into MF 8 y after and after 2 y begun RUXO to control constitutional symptoms. Both these patients started RUXO at 15 BID, but the dose was decreased after 4 weeks due to thrombocytopenia. Thereaftere, they maintained a variable dose of 5-10 BID due to hematologic toxicity. Patient 3 developed a splenic infarction after 4

weeks of treatment. No bleeding was observed during therapy. Spleen Longitudinal Diameter (SLD) was assessed by ultrasonography, performed always by the same operator. Spleen size was evaluated at baseline (BL), after 4, 12 and 24 weeks of treatment with RUXO and then every 3 months. *Results:* Trend of spleen size of our patients is reported in Figure 1. *Conclusions:* In a recent study, Pieri *et al.* demonstrate that RUXO reduces splenomegaly and local pressure in splanchnic vessels, improves splenomegaly-related symptoms and splanchnic circulation. In our experience, RUXO reduces spleen size in the first 12 weeks of treatment, even if the dose was reduced. The patient who can tolerate full dose had the major improvement. In the patients who needed dose reduction, no further decrease in spleen size was documented and a tendency to spleen re-enlargement was observed. We conclude that RUXO administered at least 15 BID efficiently reduces SLD in MPN patients with PVT, while the needing of dose reduction determines a loss of efficacy. The best effect of RUXO is observed in the first 3 months of treatment, then the spleen growth again.

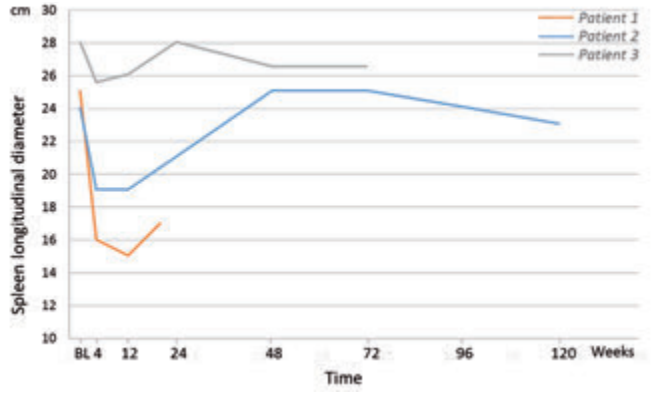

Figure 1. Trend of spleen size of our patients. Patient 1 achieved maximum SLD reduction (- 40%) from baseline (BL) after 12 weeks of treatment with RUXO at 15 BID; in Patient 2 and Patient 3 the maximum SLD reduction (respectively of -21% and -9% from BL) was observed after 4 weeks of treatment at 15 BID, no further decrease of spleen size was observed in both patients after dose reduction.

P124

A NEW METHOD FOR THE RAPID SCREENING OF CALR MUTATIONS

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The diagnosis of MPNs is based on clinical, bone marrow histology and cytogenetic criteria. According to reference WHO 2016 criteria, in patients without JAK2 and MPL mutations, the discovery of CALR mutations provides a prognostic molecular marker in ET and PMF patients Ph-. We used a new real-time PCR test (Werfen QIAGEN ipsogen® CALR RGQ PCR Kit) for the qualitative detection of somatic mutations in the exon 9 in of the gene encoding for calreticulin (CALR). The kit provides reagents to perform seven separate PCR amplification reactions in the same run for the identification of the two major CALR mutations (Type 1 and Type 2) and the detection of additional minor variants in genomic DNA extracted from human Polymorphonuclear leukocytes. To identify Type 1 and Type 2 CALR mutations, an allele-specific amplification is achieved by ARMS (Allele Refractory Mutation System) technology. For the detection of minor variants of CALR mutations, primers and probes are combined in the reaction mixes with an additional oligonucleotide that is 3'-blocked with the addition of a phosphate group (a so-called CLAMP oligonucleotide). The CLAMP oligonucleotide is specific to a wild-type targeted sequence and, when annealed, inhibits elongation of the PCR product (PCR clamping). Each CALR reaction mix includes primers and probe to detect an endogenous sequence of the ABL1 human gene. We compared Fragment Analysis with subsequent Sanger Sequencing and ipsogen® CALR RGQ PCR Kit for the detection of CALR mutations in 30 samples of MPN patients JAK2 V617F – negative. For all of these, we achieved the same qualitative data except for a positive Type 1 CALR mutation sample analysed with the new ipsogen® CALR RGQ PCR Kit. The fragment analysis of exon 9 of the gene CALR showed a low percentage of mutated CALR (7.3% of Type 1 – deletion of 52 bp) compared to wild-type genetic fragment. For this sample, the Sanger Sequencing didn't detect any mutation, providing a false negative. We hypothesize that the sensitivity of ipsogen® CALR RGQ PCR is higher than the methods used in routine laboratory tests. Therefore, we could use this method as a rapid screening tool to complete the diagnosis of MPN patients JAK2 V617F – negative, because it only takes 2 hours to evaluate 6 unknown samples and does not require further analysis to validate the type1 and 2 mutations.

P125

RECOMBINANT INTERFERON- TREATMENT AFFECTS PROGNOSIS OF LOW-/INTERMEDIATE-1 DIPSS RISK IN EARLY/PREFIBROTIC PRIMARY MYELOFIBROSIS PATIENTS: A MONOCENTRIC STUDY

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Prefibrotic Primary Myelofibrosis (pre-PMF) is a newly defined subgroup of myeloproliferative neoplasm (MPN) which mimics Essential Trombocytemia in genomic landscape and clinical presentation but differs for higher trombohemorragic and PMF evolution risk. No treatment options are currently recommended for this disease. Interferon- (INF) has already showed clonal-suppressive effect and reduction of fibrogenic milieu in Philadelphia-negative myeloproliferative neoplasms, including PMF, resulting in hematologic and molecular responses. Here, we evaluated impact of first-line IFN based-therapy on prognostic stratification and outcome in 6 pre-PMF patients treated at our clinic. Based on Dynamic International Prognostic Scoring System score (DIPSS), 3 patients were classified as intermediate-1 risk and 3 patients as low risk at diagnosis. Table 1 shows demographic and clinical data at baseline. Bone marrow biopsies revealed pre-fibrotic features and all patients fulfilled diagnostic criteria according to WHO 2016 Classification. Among int-1 risk group, one CALR type-1, one CALR type-2 and one JAK2V617F mutation were detected. One patient presented disease-related symptoms, one circulating blasts ≥1% and 2 patients splenomegaly. Patient carrying JAK2 mut was classified as Int-1 age-related. Low DIPSS risk group harbored type-1CALR (n=1) and JAK2mut (n=2) with the first showing also splenomegaly. All analyzed patients had high LDH level as well as severe piastrinosis (median value 996x10^9/L, range: 802-1491). Following treatment median time of 125 weeks (range 16-270) reduction in platelets count and spleen size were observed in all patients. Subject with high peripheral blast count achieved a reduction to <1% after 3 months of treatment and symptoms disappeared after 8 months.In Low-risk DIPSS group no patient evolved during follow up. Two patients included in DIPSS Int-1, were riassigned to low risk group with the third Int-1 patient who did not change class risk due to age (>65y). A sustained response was maintained after 165 weeks with no evidence of thrombohemorragic events. Although our analysis have been done on small size cohort of patients, it suggests IFN-alpha as advantageous treatment based on its impact on pre-PMF, carrying both type-1 and type-2 CALR as well as JAKV617F mutations with a trend toward clinical response and restaging to a lower DIPSS risk group.

P126

THERAPY WITH PEGYLATED INTERFERON ALPHA-2A IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS: LONG TERM FOLLOW-UP OF A PROSPECTIVE MONOCENTER COHORT STUDY

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Pegylated interferon alpha-2a (PegIFN2a) has been reported to be effective for cytoreduction in polycythemia vera (PV) or essential thrombocythemia (ET), and able to induce a complete molecular response of the JAK2V617F burden; moreover, it is a preferential agent in younger

patients. Fifty-seven pts. (M/F 24/33, median age 56 yrs, range 35-84) with PV (n=24), ET (n=32), myelofibrosis (n=1) treated with PegIFN2a were prospectively evaluated from July, 2010 to April, 2017. All patients but two with ET had the JAK2V617F mutation. Hematological response was assessed according to the ELN criteria. The JAK2V617F allele burden was tested before starting treatment and then every 3 months, being evaluable for molecular response if quantitated on at least two different occasions. Twenty-three pts. received PegIFN2a as a first line treatment, and 34 after discontinuation of previous treatment. The median dosage was initially 90 mcg/ week (range 67-180), and after adjustement for adverse effects and response 67 mcg/week (range 15-360). The median duration of treatment was 182 (range 4-320) and 34.5 weeks (range 5-304) in PV and ET, respectively. Hematological response was complete in 15 (26%, PV=6, ET=9) and partial in 17 pts. (30%, PV=4, ET=13). The baseline median JAK2V617F allele burden of the evaluable patients was 80% (range 16-100) in PV (n=19) and 26% (range 6-98) in ET (n=25); PegIFN2a reduced the allele burden in PV (median 49%, range 0-100, p=0.048), but not in ET (median 27%, range 4-100, p=0.77); in 8 pts. (44%) JAK2V617F was decreased > 50%, and in one became undetectable. Adverse events occurred in 42 pts. (74%): flu-like symptoms (n=18), thyroiditis (n=11), itching (n=9), atrial fibrillation (n=5), elevated liver enzymes (n=4), diarrhea (n=2), depressive state (n=2), and pulmonary fibrosis (n=1). Treatment was discontinued for toxicity in 36 pts. (63%) and in 5 pts. (9%) for inefficacy. Thirty-two pts. had a previous thrombosis (56%); while on PegIFN2a, 8 pts. (14%) had a recurrence (n=5, incidence 6.4 per 100 pt-yrs) or a first event (n=3, incidence 7.1 per 100 pt-yrs), in spite of antithrombotic prophylaxis. In conclusion, PegIFN2a was effective in inducing hematological response in PV and ET patients, obtaining a molecular response in at least one-third of PV patients, but not in ET. However, the rate of discontinuation for toxicity was higher than previously reported. Finally, efficacy of PegIFN2a in preventing thrombosis was unsatisfactory.

Hodgkin Lymphoma

P127

GONADAL FUNCTION RECOVERY AND FERTILITY IN WOMEN TREATED WITH CHEMO ± RADIOTHERAPY FOR HODGKIN'S AND NON HODGKIN'S LYMPHOMAS

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Background: Significant improvement in the survival rates of patients with Hodgkin's (HL) and Non Hodgkin' Lymphomas (NHL) has been achieved in the last decades; however, among the adverse events, loss of fertility and gonadal function failure represent two of the most important aspects for young women. Purpose: The aim of this study was to assess the menstrual status and fertility in patients treated for HL and NHL after at least 5 years of follow-up. *Patients and methods:* Female patients in remission since at least 5 years from the end of chemotherapy±radiotherapy were requested to respond to a questionnaire. Menstrual status, treatment with oral contraceptives or GnRH (gonadotrophin-releasing hormone) analogues during radio-chemotherapy and pregnancies from the end of the therapeutic program were evaluated. As for their influence on amenorrhea the following factors were evaluated: age, chemotherapy regimen, the use of radiotherapy and the use of oral contraceptives (OCs) or GnRH analogues during chemotherapy. *Results:* A total of 97 patients with HL or NHL answered to the questionnaire. The median observation time after treatment was 12 years. The resumption of menstrual activity resulted associated with the use of the OCs and GnRH analogues during chemotherapy (P= 0.008 and 0.034 respectively). At univariate analysis the use of OCs during chemotherapy was associated with a lower risk of secondary amenorrhea (PR=0.37; 95% CI 0.17-0.82). A higher age at the time of treatment correlated positively with therapy-induced amenorrhea (P=0.000); in particular,we observed a difference of 12.8 years at diagnosis between the mean age of women with therapy-induced amenorrhea and those who resumed their menses. Moreover, amenorrhea was significantly higher in women receiving R-CHOP than in women treated with ABVD (PR=6.00; 95% CI 2.32-15.54). Premature menopause was experienced by 9% of women, while 19% had children after therapy, with an average of 1.33 births per women. *Conclusions:* This study provides clinically important information about fertility and late ovarian function impairment in long-term survivors of HL and NHL and suggests a possible role of pharmacological prophylaxis with OCs and GnRH analogues in restoring gonadal function.

P128

BRENTUXIMAB VEDOTIN AS SALVAGE TREATMENT IN HODGKIN LYMPHOMA NAÏVE TRANSPLANT PATIENTS OR FAILING ASCT: THE REAL LIFE EXPERIENCE OF RETE EMATOLOGICA PUGLIESE (REP)

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Background: Brentuximab vedotin (BV) showns a high overall response rate (ORR) in relapsed or refractory (R/R) Hodgkin Lymphoma (HL) after autologous transplant (ASCT).More recent data suggest the

role of BV as a bridge therapy to ASCT and allogeneic transplant (alloSCT). *Patients and Methods:* An observational,multicenter, retrospective study was conducted in nine Hematology Departments of Rete Ematologica Pugliese, to analyze outcome in term of ORR, PFS and OS and toxicity of BV as salvage therapy and as bridge regimen to ASCT or alloSCT. Seventy patients received BV at standard dose. 45 patients (64%) were treated as bridge to ASCT (16;23%) or to alloSCT (29,41%).Twenty-five (36%),not elegible for transplant, received BV as salvage treatment. Baseline patients characteristics are summarized on table1. No differences in baseline characteristics were observed between the different cohorts of patients. *Results:* The ORR was 59% (CR 26%). The ORR in transplant naïve patients was 75% (CR 31% and PR 44%). In patients treated with BV as bridge to alloSCT, the ORR was 62% CR 24%; PR 38%), table 2. Univariate analyses revealed that the ORR was lower in patients with early first-relapse (45%*vs*76%; p<0,03), in refractory patients (29%*vs*72%; p<0.005) and in patients treated with BV after 17months post diagnosis (39%*vs* 65%; p<0,05). In multivariate analysis the only prognostic variable was refractory disease(p<0,005). The 2y-OS in whole population was 70%. The median PFS was 17 months. The 2y-PFS was lower in early relapse patients (43% *vs* 82%; p<0.04) and in refractory disease (48% *vs* 73%;p<0.03), Figure3. At the time of this analysis, ten of 16 naïve-transplant patients (63%) received ASCT, and 50% achieved CR before ASCT. The ORR at +90-post ASCT was 90%(40% CR) and 2y-PFS was 81%. In the 29 pts treated with BV as bridge to alloSCT, 28 (97%) proceeded to alloSCT with 25% in CR prior alloSCT. The ORR at +90 was 52%(30% CR and 22% PR). The 2y-PFS was 68%.The most common treatment-related adverse events were peripheral neuropathy (50%),neutropenia (29%),anemia (12%). *Conclusions:* These data suggest that BV is well tolerated and very effective in R/R HL patients, producing a substantial fraction of CR. BV may also be a key therapeutic agent to achieve a good disease control before ASCT or alloSCT, improving post transplant outcomes, also in refractory and heavily pretreated patients, without significant overlapping toxicities with the prior therapies.

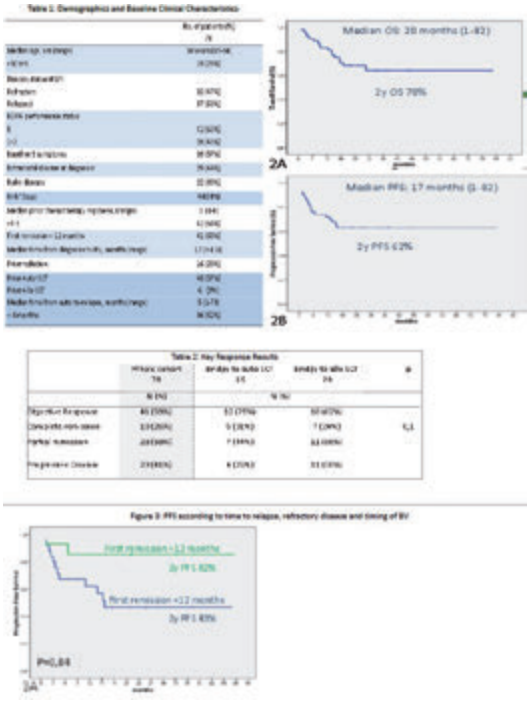

Figure 1.

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SURVIVORSHIP PROGRAM: SECOND MALIGNANCIES IN LYMPHOMA SURVIVORS

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Background: Improvements in the treatment of both Hodgkin's (HL) and non Hodgkin's Lymphomas (NHL) have resulted in an increasing number of long term survivors. However this patient's population is at high risk of developing late therapy related complications that can negatively affect long term survival and quality of life. *Aims and Methods:* In our institution the HL and aggressive NHL long term survivors are followed up in a dedicated clinic since September 2014. Here we report data on second malignancies diagnosed after lymphoma resolution. We have collected retrospective data on second tumours in 548 consecutive lymphoma survivors. *Results:* We have analyzed data regarding 548 patients coming in our clinic from 15 September 2014 to 20 April 2017, 273 have been successfully treated for HL and 275 for NHL. Two hundred seventy were females, 278 males; median of age at lymphoma diagnosis was 29 years for HL (range 13-84) and 48 years for NHL (range 12-83). Seventy eight patients (14%) experienced a second cancer, 8 of them had 2 neoplasms, so we documented 86 second tumours. They were: 31 skin (36%) and 55 non cutaneous cancers (64%). Two of the cutaneous neoplasm were melanomas.The non skin neoplasm were: 15 breast, 9 gastroenteric, 10 thyroid, 6 prostatic, 1 lung, 5 bladder, 1 renal, 1 tongue, 1 testis, 2 ovarian, 1 laringeal, 1 meningioma, 1 myelodysplastic syndrome with excess blasts and 1 cutaneous appendages malignant cancers. One patient, with esophagus carcinoma, has dead few weeks after diagnosis. Six of these tumours (2 colon, 2 breast, 1 thyroid and 1 ovarian) have been diagnosed with our program of early diagnosis of second cancers and thyroid dysfunctions. The median of time between diagnosis of lymphoma and diagnosis of second malignancy was 18 years (range 1-41). Regarding the previous therapies: 25 patients had received radiotherapies in the site of secondary tumours. (32%). Moreover we have documented relapse of their original lymphoma in 4 patients (1 HL and 3 nHL). *Conclusions:* In our Department we described a significant number of cases of second neoplasms in the lymphoma survivors population: 6 of these were detected by tests done for early diagnosis of late complications. These results outline the importance of a risk adapted plan for early diagnosis of cancers in this setting of patients that would be encouraged by both hematologist and general practitioners.

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IMPACT ON SURVIVAL OF EARLY DETECTION OF RECURRENCE IN THE FOLLOW-UP OF HIGH RISK HODGKIN LYMPHOMA IN FIRST COMPLETE REMISSION

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Introduction: Despite the high complete response (CR) rate to first-line therapy, about 1/3 of patients with advanced-stage Hodgkin lymphoma (HL) relapses. Many relapses are clinically asymptomatic and a close monitoring, based on imaging procedures is justified if an early detection of recurrence would allow a timely administration of salvage therapy and a survival improvement. *Aim:* The purpose was to evaluate the response to salvage therapy of relapsed HL by comparing patients who received surveillance with conventional clinical assessments *vs* imaging procedures. The primary end-point was to assess the CR rate to salvage therapy at first relapse. Secondary endpoints were: the recurrence rate after first CR, the stage and extranodal involvement at relapse, and the disease-free survival (DFS). *Methods:* Between June 2001 and December 2009, we analyzed 306 patients with high-risk HL in first CR. In this case-control study, the first cases (n=156) consisted of patients who received a conventional follow-up program including symptom assessment, blood tests and physical examination (Historical cohort-HC). Subsequent patients (n=150) received routine imaging procedures comprising ultrasonography (US) of superficial, anterosuperior mediastinal, abdominal, and pelvic lymph nodes (SMAP-US), and chest radiography (CXR), as integrated part of the follow-up (Imaging group-IG). Follow-up procedures were performed at 4-8-12-16-20-24-30-36-48-60-84 and 108 months. Relapses were documented by histology. At relapse all patients received salvage therapy (DHAP), for at least two courses, followed by ASCT. *Results:* After a median 62-months observation (range, 4–108), 83 patients, evenly distributed in the two groups, relapsed. Of these, 29 of 43 patients (67.4%) of the HC *vs* 17 of 40 patients (42.5%)

of the IG, showed stage superior to IIB at restaging (p=0.02) and a more frequent extranodal involvement, 23.3% in the HC *vs* 7.5% in the IG (p=0.01). Asymptomatic relapses were detected in 26/43 (60.4%) patients in the IG and 17/43 (39.5%) patients in the HC, p=0.02. CR rate with second line therapy was higher in the IG (27, 67.5%) compared with the HC (19, 44.2%; p=0.032). The 3-years DFS was 75% in the IG and 36% in the HC, p=0.02. *Discussion:* This is the first prospective case-control study using SMAP-US+CXR to monitor patients with advanced stage HL. Our data indicate that the early detection of HL recurrence allows to begin rescue therapy in patients with a more limited disease and, consequently, increase its effectiveness in terms of probability to response and DFS.

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ULTRASOUND ELASTOSONOGRAPHY: A USEFUL TOOL FOR THE CHARACTERIZATION OF SUPERFICIAL LYMPHADENOPATHIES SUSPECTED FOR LYMPHOMA

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Introduction: In patients with suspect neoplastic diseases, lymph-node (LN) ultrasonography (US) with Doppler evaluation of hilar arterial vascularization proved to be a powerful non-invasive tool to investigate lymphadenopathies. However, differential diagnosis could be difficult in some situations. The recent introduction of US elastography (ES) to measure tissue stiffness has increased the capability of conventional US for the early diagnosis of breast and thyroid cancer. In particular, quantitative ES, especially with strain ratio (SR) index, improves diagnostic accuracy for malignancy. *Aim:* This prospective study aims to evaluate the diagnostic efficacy of real time ES in differentiating benign from malignant lymphadenopathies. *Methods:* We evaluate enlarged LNs of 58 patients with suspect diagnosis of lymphoma, were examined using Power Doppler US scan followed by ES. The following LN features were considered for malignancy diagnosis: (a) long axis greater than or equal to 1.5cm, (b) round shape, (c) hilus absent, (d) hypoechoic parenchyma, and (e) hypervascularization, (ie, intranodal arterial vessels with high resistive index value [≥0.6]). In all patients, we measured LN stiffness using a four points scale and the muscle-to-LN SR. After combined US+ES examination, each lymph node was undergone histological examination. We evalueted specificity and sensitivity of stiffness measurement both with four points scale and SR. *Results:* The pathologic evaluation of lymph node biopsies provides the following diagnosis: 24 benign hyperplasia, 15 Hodgkin Lymphoma (HL), 6 DLBCL, 4 SLL/CLL, 3 follicular lymphomas, 2 T-cell lymphomas, 1 marginal zone lymphoma, 1 mantle cell lymphoma and 2 metastatic carcinomas. Measuring LN stiffness using a four points scale, we obtained a 73.3% specificity and a 70.4% sensitivity (p=0.005). Instead, using SR, the overall diagnostic efficacy for malignancy of the Power Doppler US followed by ES indicated a 92% specificity and a 79% sensitivity; median SR was 0.88 (range 0.30-2.03) for benign lymphadenopathies and 1.96 (range 0.57-3.27) for malignancies. Sensitivity and specificity were respectively 93% and 92% for HL. *Conclusions:* ES is a simple, fast and non-invasive diagnostic method that may be a useful aid to US in the assessment of lymphadenopathies in patients suspected of having lymphoma. The high specificity of this test in all lymphomas subtypes, in particular using the SR calculation, could help to avoid unnecessary LN biopsy in patients with non-malignant lymphadenopathies.

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LONG-TERM RISK IN THE ERA OF MODERN THERAPY FOR HODGKIN LYMPHOMA. RETROSPECTIVE STUDY IN 295 HODGKIN'S LYMPHOMA PATIENTS TREATED FROM 2008 TO 2011

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Introduction: Long-term survivors of Hodgkin lymphoma (HD) experience several late adverse effects of treatment, with cardiovascular diseases and second malignant neoplasms being the leading causes of death. *Patients and Methods:* In this study we assessed the incidence of late therapy-related side effects (second malignancies, cardiovascular diseases and endocrinopathies) in our center. HD patients (pts) treated between 1999 and 2011 were followed over the time.Results We have enrolled 295 pts. 293/295 pts were treated with chemotherapy (CHT) followed by radiation therapy (RT); ABVD regimen was the most widely used regimen with 287 pts treated, while 6 pts received a different CHT. In only 2 cases the first-line therapy was RT alone.At the end of the study (August 22, 2016), 255 pts were alive (86%), while 21 were dead (15 pts of HD, 4 due to a second neoplasia, 1 following a heart attack and another acute toxicity therapy). Pts who had RT in the front line were 165 (56%). 12/295 pts (5%) developed solid cancer (3/12 breast neoplasm) and 4/295 (1%) developed second hematological malignancies. 16 pts (5%) experienced cardiac toxicity (50% valvulopathie and 50% heart attack) ,46 (16%) experienced at least endocrinopathy (7% amenorrea).When pts who had RT were compared with pts who had not made RT, the risk of second malignancy (p 0,005), heart disease (p 0,009) and endocrine disorders(p 0,000)was significantly higher. We have divided pts in 2 groups according to the treatment period. Group 1: pts treated from 1999 to 2007 with of extended-field or mantle RT (40-46 Gy); group 2: pts treated from 2008 to 2011 with involved-field RT (IFRT) (30-36 Gy). In the first group of pts there was a higher risk of late toxicity (p=0.05 for second solid tumors; p=0.000 for heart disease; p=0.05 for endocrine disorders). There are no significant differences between the 2 groups in terms of survival or response to therapy. Overall survival after a median follow-up of 107 months (range 1-214) was 95%and progression-free survival was 89% at 103 months (range 1-124). *Conclusions:* Pts who undergo RT during the first-line therapy for HD have a higher risk of late toxicity when compared to pts who do not RT. IFRT reduces irradiated volumes and produces significant reductions in normal tissue dose compared with historic treatments. In the future the introduction of involved-node RT and response-adapted therapy could result in a reduction in long-term toxicity.This work was supported by the Legato Zottola Donation and Fondazione Biagioni Borgogni O.N.L.U.S.

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RITUXIMAB IN A RISK-ADAPTED TREATMENT STRATEGY GIVES EXCELLENT THERAPEUTIC RESULTS IN NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA

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Nodular lymphocyte-predominant Hodgkin lymphoma (NLP-HL) is a rare variant of HL. Due to its rarity, consolidated and widely accepted treatment guidelines still lack for this disease. The expression of CD20 on neoplastic lymphocytes provides a suitable target for novel treatments based on Rituximab (R). Twelve consecutive newly diagnosed adult patients with NLP-HL received R alone or combined with ABVD (Doxorubicin, Bleomycin, Vinblastine, Dacarbazine) according to the baseline risk. The five patients with early favorable disease received R as single agent, once per week for four weeks, followed by R maintenance (once every three months for 2 years). The two patients with early unfavorable disease received R once per month for 4 months plus 4 ABVD cycles, while the remaining five patients with advanced-stage received R twice a month for 6 months plus 6 ABVD cycles. The treatment efficacy and safety were compared to those of a historical cohort of 12 consecutive patients with NLP-HL who received 4 ABVD courses followed by involved-field radiotherapy if at stage I or II (n=9), or 6 ABVD courses if at stage III or IV (n=3). After 4.3-year follow-up, the K-M estimates of progression free survival were 100% for the patients treated with R-containing regimen *versus* 66% for the patients of historical cohort (p= 0.04 - Figure). Four patients in the latter group showed insufficient response to therapy: one relapse in the early-stage sub-set, and one refractory and two relapses in the advanced-stage sub-set were recorded. Over the study period, one patient died for pneumonitis following the last cycle of R-ABVD. No patient developed a secondary malignancy. Our results confirm the value of R in NLP-HL and show

that R induction and maintenance, with chemotherapy only in presence of risk factors or advanced-stage, give excellent results compared to conventional chemo-radiotherapy, while sparing cytotoxic agent and/or irradiation-related toxicity.

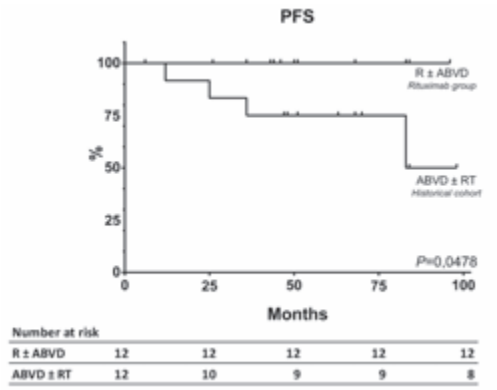

Figure 1.

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BONE INVOLVEMENT IN HODGKIN'S LYMPHOMAS. A SINGLE CENTER EXPERIENCE

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Hodgkin's lymphoma (HL) is a highly curable disease and 5-year survival is improving, being currently 86%. Extranodal involvement is a common feature of malignant lymphomas, especially non-Hodgkin lymphomas, which include as many as 30% of extranodal origin. In HL the incidence of extranodal disease is somewhat lower; the spleen is most frequently involved (up to 25% in mixed cellularity subtype) but extralymphatic organs involvement is far less common. Extranodal involvement is a prognostically unfavorable feature in HL. In the initial workup, bone marrow, liver and lungs are evaluated for possible involvement; bones are considered a very rare extranodal site of HL. The purpose of the study was to determine the incidence and prognostic impact of bone involvement in HL. We performed a retrospective single institution study of 341 cases (207 (61%) males and 134 (39%) females) with a median follow-up of 44 months. Median age at diagnosis was 36 years (range 15-83), 106 pts (31%) had advanced stage disease (III-IV), 128 (38%) bulky disease, 161 (47%) presented B symptoms, 92 (27%) spleen involvement. The histology was nodular sclerosis in 287 (84%). *Results:* 55 patients (16%) had extralymphatic disease, 24 (7%) had primary bone involvement, always with concurrent nodal disease. 14 (58%) had B symptoms, 9 (37%) had bulky disease, 19 (79%) had stage IV disease. The bone involvement subset presented more frequently with advanced stage disease (91% *vs* 21% p<0.001), a significantly higher (>2) HD-score (66% *vs* 10% p<0.001), B symptoms (58% *vs* 46% p=0.02), and a higher ESV level (83% *vs* 37% p=0.001). The bone involvement group had poor prognosis compared with those with no bone involvement. (5 year progression-free survival [PFS], 73% *versus* 82%; p=0.010). Complete remission (CR) rates in the bone and no bone involvement patient subsets were 56% *vs* 71% (p=0.003), respectively. *Conclusions:* In our study, bone involvement in pts with HL was a rare occurrence (7%) associated with a poor clinical outcome.

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2-[FLUORINE-18] FLUORO-2-DEOXY-D-GLUCOSE-POSITRON EMISSION TOMOGRAPHY/WHOLE-BODY MAGNETIC RESONANCE IMAGING (FDG-PET/MRI), INCLUDING DIFFUSION-WEIGHTED IMAGING (DWI), FOR STAGING PATIENTS WITH CLASSICAL HODGKIN LYMPHOMA (C-HL) AND DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL): COMPARISON WITH FDG-PET/CONTRAST-ENHANCED COMPUTED TOMOGRAPHY (CT) IN A PROSPECTIVE STUDY

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DLBCL and c-HL require accurate staging in order to planan appropriate therapy; CT, together with FDG-PET, is fundamental for this purpose. Because of the increased survival rates of patients with c-HL and DLBCL, the goal of current therapies is to maximize cure rates while minimizing toxicity. In line with this paradigm, avoiding CT-related ionizing radiation exposure and i.v. contrast medium-related kidney toxicityis desirable. Unenhanced whole-body MRI is feasible and may be a good radiation-free alternative to CT for staging lymphoma. The aim of this study was to compare staging obtained with fused FDG-PET/MRI with staging obtained with FDG-PET/diagnostic CT for patients with newly diagnosed lymphoma (clinicaltrials.gov registration no.NCT03042247). At pretreatment staging, all patients with c-HL and DLBCL underwent same-day FDG-PET/contrast-enhanced CT and FDG-PET/whole-body MRI (Biograph mMR imager; Siemens Healthcare, Erlangen, Germany) with DWI. Nodal and extra-nodal involvements were evaluated site by site using qualitative and quantitative image analysis and interpreted by consensus of radiologists and nuclear medicine physicians. Overall, 30 consecutive patients with newly diagnosed lymphoma were scheduled to receive imaging tools for staging. Of them, 7/30 (23.3%) failed MRI examination because of claustrophobia, thus 23 patients were analyzed. The percentages of agreement between FDG-PET/MRI and FDG-PET/CT were 100% for all nodal regions (, 1.00) and 96% for extra-nodal regions (, 0.69). In particular, FDG-PET/MRI showed a significantly higher overall diagnostic accuracy than FDG-PET/CT to detect bone focal lesions (100% *vs*78%, respectively). Ann Arbor stages according to FDG-PET/MRI were concordant with those of FDG-PET/CT in 100% (23/23) of patients. The average dose of ionizing radiation and of i.v. nonionic contrast medium (diagnostic CT) employed was 19.9 mSv (range, 13.9-25.8) and 140 ml (range, 120-150), respectively. The results of this study suggest that fused FDG-PET/MRI equals FDG-PET/diagnostic CT for staging patients with newly diagnosed c-HL and DLBCL. Moreover, MRI scans allow a more precise assessment of bone infiltration by lymphoma. Whole-body MRI with DWI can be a good alternative to diagnostic CT when radiation exposure and i.v. contrast medium need to be avoided.

Table 1. Region-based lymphoma assessment on FDG-PET/CT and FDG-PET/MRI.

Positive regions*			
	Nodal	Extra-nodal	Total
PET/CT	74	11	85
PET/MRI	74	14	88
Region-based agreement*			
	Nodal *κvalue*	Extra-nodal *κvalue*	
PET/CTvsPET/MRI	1.00	0.69	

*Total number of regions for comparative analysis
FDG indicates fluorodeoxyglucose; PET, positron emission tomography; CT,computed tomography;MRI, magnetic resonance imaging

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PRE-TRANSPLANT POSITRON EMISSION TOMOGRAPHY (PET) PREDICTS RELAPSE RATE IN PATIENTS WITH HODGKIN LYMPHOMA UNDERGOING HAPLO TRANSPLANTS WITH POST-TRANSPLANT CYCLOPHOSPHAMIDE

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Background: Patients with Hodgkin Lymphoma (HL) relapsing following autologus stem cell transplantation (autoSCT) have an overall poor prognosis, with a reported median survival ranging between 7.3 and 25 months [Mosckowitz et all. BHJ 2009]. Although reduced intensity allogeneic transplant (RIC alloSCT) is considered a feasible option for

those patients [Luznik *et al.* BBMT 2008], relapse remains a significant cause of allo-SCT failure. In this scenario, identification of prognostic factors associated with RIC alloSCT outcomes is the first step for any strategies aimed to decrease the risk of relapse. *Methods:* Forty-four consecutive HL patients received unmanipulated haploidentical bone marrow RIC alloSCT with post-transplant cyclophosphamide (PT-Cy) (Raiola *et al.* BBMT 2014) between September 2009 and June 2015 at our institution. Residual metabolic activity before alloSCT was assessed by positron-emission tomography (PET). Deauville 5-points score was used to interpretate the results of pre-transplant (PET) scans, with a threshold of 4 or above considered positive. Cox univariate and multivariate regression model was performed to identify pre-transplant factors influencing outcomes. *Results:* All patients had received at least 3 chemotherapy lines and all but one an autoSCT. Median age was 31 years (range 18-60). All patients engrafted but 1 who showed autologous recovery. Cumulative incidence of grade II-IV acute GvHD and 3-year moderate-severe chronic GvHD were 18.6% and 11.6%, respectively. With a median follow-up of 32 months (range 16-85), 21 patients relapsed (47%). All relapsed patients were treated with further therapy, mainly immuno-chemotherapy and donor lymphocyte infusion (DLI). The 3-year overall (OS), progression free survival (PFS) and graft relapse free survival (GRFS) were 77%, 66%, and 41%, respectively. The 3-year non-relapse mortality (NRM) was 5%. On multivariate analysis pre-transplant PET status, available in 40 of 43 engrafted patients, was the only factor determining outcome (relapse rate HR=3.6; 95%CI: 1.4-9.4; p=0.01; 3-year PFS HR=3.8; 95%CI: 1.1-13.6; p=0.04). Outcomes accordingly to pre-transplant PET status are shown in the following table. *Conclusions:* Pre-transplant PET status is a prognostic factor of HL submitted to RIC haplo SCT. Patients with pre-transplant PET Deauville score of 4 or above may be possible candidate to pre- or post- alloSCT experimental strategies.

Table 1.

Pre-transplant PET status (n of pts)	3 year Relapse Rate	Grade II-IV aGvHD	3 year cGvHD	3 year PFS	3 year OS	3 year GRFS
Positive (22)	66%	27%	11%	41%	64%	24%
Negative (18)	27%	11%	17%	81%	89%	58%
p-value	0.01	0.41	0.47	0.04	0.12	0.06

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BRENTUXIMAB VEDOTIN FOR THE TREATMENT OF HODGKIN LYMPHOMA AND CD30+ NON HODGKIN LYMPHOMA: A MULTICENTER TUSCANY EXPERIENCE OUTSIDE CLINICAL TRIAL

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Background: Brentuximab Vedotin (BV) showed a high overall response rate in refractory/relapse Hodgkin lymphoma (HL) and CD30+ non Hodgkin lymphoma (NHL). This is a multicenter observational retrospective analysis of patients affected by HL or NHL who were treated with BV until disease progression or unacceptable toxicity. The aim of this study is to evaluate the efficacy and safety of BV outside clinical trial and to identify the time of the best response rate. *Patients and Methods:* we collected 82 consecutive patients treated in Tuscany with BV from 2013 to 2017: 68 pts affected by HL and 14 pts with NHL. All pts had histologically confirmed CD30+ disease. The median age was 43 years for HL (range 16-86) and 68 for NHL (range 17-78). Among HL there were 21 (31%) stage I-II cases and 47 (69%) stage III-IV; B symptoms were present in the 60% of cases. NHL included 10 ALCL, 2 DLBCL,1 grey zone lymphoma, 1 gd cutaneous. Fourty-five pts (66%) for HL and 9 pts (64%) for NHL were refractory to the first line treatment. Median number of prior treatments was 2 (range 0–7) including prior ASCT in 33 cases (48.5%) for HL, 1(range 0-7) including 1 prior allogenic transplant and 2 ASCT for NHL. *Results:* The median number of BV infusion was 7 (range 1-16) for HL and 5 (range 1-16) for NHL. Among HL 25 patients obtained a CR (36,8%) and 15 patients a PR (22%) with an overall response rate of 58.8%. For NHL we described 4 CR (28,6%) and 1 PR (7%) with an ORR of 35,6%. The median best response overall was obtained at 4 cycle (range 2-12). The median DOR for patients who achieved at least PR was 18,7 months. The OS from the first dose of BV was 54% at 38 months (median not reached). Generally, the therapy was well tolerated also by elderly pts. We observed 3 cases of hematological toxicities (grade 3 and 4 thrombocytopenia and grade 3 neutropenia) and 9 cases of peripheral neuropathy gr. 2-3 for which 3 pts discontinued the treatment. One pt discontinued the BV infusion due to cutaneous rash and infusion reaction despite of premedication. No other toxicities were observed during the follow up. *Conclusions:* Our study describes the use of BV in the real clinical practice and confirm the high effectiveness and small number of adverse event even in heavily pre-treated and elderly pts. Furthermore it leads to a rapid response and it can be use as a bridge to ASCT or HSCT.

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HISTOLOGICAL VALIDATION BY IMAGING-GUIDED CORE-NEEDLE-CUTTING BIOPSY OF 4 TO 5 POINT SCALE DEAUVILLE CRITERIA: RETROSPECTIVE ANALYSIS IN HODGKING LYMPHOMA AT RESTAGING

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[18]Fluorodeoxyglucose positron emission tomography combined with computed tomography (FDG PET-CT) is fundamental in staging and post-treatment restaging of Hodgkin lymphoma (HL). At the end of induction chemotherapy, it is necessary to distinguish the responders from the non-responders. The Deauville criteria (DC) recommends a 5-point scale rather than taking a binary decision (PET positive or negative). In particular, an accurate judgement of residual lesions scored as DC 4 or 5, is crucial to define salvage chemotherapy with autologous stem cell transplantation (SCT). We retrospectively evaluated post-induction chemotherapy restaging including FD GPET-CT in a group of HL patients. The positive predictive value and specificity of DC were assessed by using histological examination; in particular, in our Institution all residual lesions scored as DC 4 or 5 systematically underwent imaging-guided core-needle-cutting biopsy (CNCB). We included in this retrospective study all FDG PET-CT examinations performed on 194 patients at restaging after front-line therapy with ABVD for HL between January 2008 and December 2016 at the Hematology Department of Federico II University of Naples. We considered for our study only 4-5 scored exams. Of all patients, 23/194 (11.8%) had a DC 4 and 13/194 (6.7%) a DC 5. For these patients, with positive PET findings, histological clarification by ultrasonography or CT-guided CNCB was scheduled in order to define further therapy. Thereafter histological examination to clarify the persistently high uptake was performed in 34/36 patients with 4-5 DC. On the basis of histological findings, 10/34 FDG PET scans were categorized as true positive, 22/34 FDG PET scans were depicted as false positive, while in 2/34 cases the core-needle samples were inadequate and these patients were excluded. Thus, 32 patients were assessed for the final analysis. The positive predictive value of 4-5 DC was 0.31, with a specificity of 0.87. According to our Institution strategy, patients resulting positive from CNCB were treated with a salvage scheme (chemotherapy and SCT), while for patients with negative histological results radiotherapy as previously planned or a watch and wait approach was performed. After a median follow-up of 24 months, further PET examinations showed no pathological uptake in the 22 patients with false-positive lesions. Further prospective multicenter studies are required to obtain histological validation of DC score, especially in the real-life.

Table 1.

Deauville score at restaging after front-line therapy with ABVD	Number of patients	Number of patients excluded from analysis	Number of true positive cases	Number of false positive cases	Specificity (95% CI)	Positive Predictive Value (95% CI)
4 (moderately >LIVER)	23	2	3	18	0,8953 (0,8397-0,9368)	0,1429 (0,03049-0,3634)
5 (markedly >LIVER and/or new lesion)	13	2	7	4	0,9747 (0,9364-0,9931)	0,6364 (0,3079-0,8907)

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BRENTUXIMAB VEDOTIN PLUS HIGH-DOSE BENDAMUSTINE IS EFFECTIVE AND HAS A FAVOURABLE TOXICITY PROFILE IN THE TREATMENT OF REFRACTORY OR RELAPSED HODGKIN LYMPHOMA

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The objective of this retrospective observational trial was to evaluate efficacy and safety of salvage cytotoxic regimens in patients with refractory or relapsed HL. 32 consecutive patients (19 M/13 F) with classical HL and median age of 31.7 years (r.16-73) received a salvage regimen after failure of ASCT. Patients were by chance assigned to one of these three arms: standard dose bendamustine (90 mg/sqm) days 1 and 2 plus standard DHAP schedule (every 4 weeks) x 3 cycles (Arm A, n= 10), brentuximab single agent 1.8 mg/kg (every 3 weeks) x 4-8 cycles (Arm B, n=11), and high-dose bendamustine (120 mg/sqm) days 1 and 2 plus brentuximab 1.8 mg/kg (day 3) x 4-6 cycles (Arm C, n= 11). The treatment efficacy in each arm was evaluated according to Revised Response Criteria for Malignant Lymphoma. Any adverse event occurred was recorded and classified for type and grade using NCI-CTCAE criteria (v 4.0). In arm A, ORR was 40% (4/10), with 4 (40%) CR and 6 (60%) PD. Hematological toxicity was grade 3 thrombocytopenia in 4 patients (40%) and bone marrow aplasia in 1 patient (10%); extra-hematological toxicity was gastrointestinal toxicity of grade 2 in 6 patients (60%) and grade 1 in 3 patients (30%). In arm B, ORR was 63.6% (7/11), with 5 (45%) CR, 2 (18%) PR and 4 (36%) PD. Hematological toxicity was grade 2 neutropenia in 4 patients (36%), extra-hematological toxicity was grade 3 neuropathy in 2 (18%). In arm C, ORR was 100% (11/11), with 11 CR followed by SCT (second autologous transplant, 6; or haploidentical transplant, 5) with persistence of CR in all patients at a median follow-up of 33.4 months (r. 12-60). Hematological toxicity was grade 3 thrombocytopenia in 4 patients (36.3%); extra-hematological toxicities were increase of transaminase (grade 2) in 3 (27%) and CMV reactivation in 2 (18%), treated successfully with valganciclovir. Three patients had fever during infusion at first cycle, together with a skin rash, managed with corticosteroid injections, and a successful antihistamine plus corticosteroid prophylaxis in the next cycles of treatment. High-dose bendamustine plus brentuximab has shown relevant efficacy and a relatively good safety profile in a setting of heavily pretreated patients with HL. Adequate monitoring of CMV reactivation is recommended. This combination could be considered as a bridge to second autologous or allogenic SCT. However, these results should be validated by controlled and prospective studies involving larger number of patients.

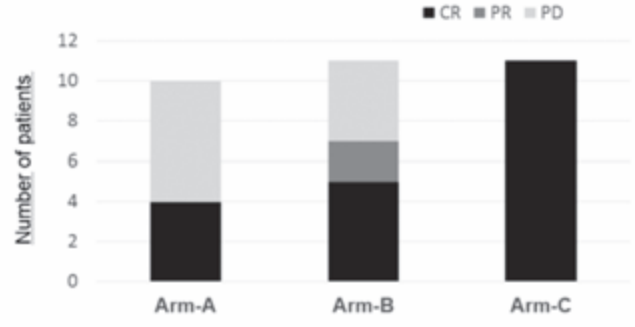

Figure 1.

Non Hodgkin Lymphomas 1

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LIQUID BIOPSY: DECIPHERING A SIGNATURE OF CIRCULATING MICRORNAS AS NOVEL NON-IVASIVE BIOMARKERS IN DIFFUSE LARGE B-CELL LYMPHOMA. PRELIMINARY RESULTS OF A PROSPECTIVE STUDY

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Bodily fluids are important source of information in several diseases analyzable by liquid biopsies, representing minimally invasive methods for diagnostics and prognosis. Blood extracellular miRNAs are under investigation as novel biomarkers. While tissue miRNAs in DLBCL patients have been extensively studied, only few reports evaluated the role of circulating miRNA as potential prognostic factors. Our aim is to identify and validate a serum miRNA signature with prognostic value in DLBCL. This is a on-going prospective study on newly diagnosed DLBCL patients uniformly treated with 6 courses of R-CHOP. Serum samples of patients were collected at diagnosis and after the end of treatment. The expression profile of selected circulating miRNAs described as associated with lymphoid malignancies by us (miR-22 and let-7c/miR-99a/miR-125b cluster) and by previously published studies (miR-22, miR-18a and miR-20a) was evaluated by qRT-PCR in serum samples collected at diagnosis of the first 18 patients enrolled into the study. Our results showed that serum miR-22 expression as well as let-7c/ miR-99a/125b cluster was significantly higher at diagnosis in patients unresponsive to treatment when compared with responsive patients. On the contrary, miR-18 and miR-20 levels appeared to be not significantly associated to treatment response. In addition, a global expression profile of circulating miRNAs was evaluated in serum samples derived from a smaller cohort of patients. We found a striking difference in miRNA modulation upon treatment between unresponsive and responsive patients. In particular, we found 31 miRNAs significantly modulated after R-CHOP in the group of responsive patients, including miR-22. In contrast, this miRNA subset did not show expression changes in unresponsive patients. Moreover, we performed a study interrogating The Cancer Genome Atlas database. We found that the only available data are relative to the miRNA expression levels in tumor tissue samples of 47 DLBCL patients, finding a signature of 13 miRNAs with potential prognostic value. Among these we found that miR-22, emerged as modulated in our genome-wide analysis, was linked to risk of disease recurrence. These preliminary data suggest that the serum miR-22 as well as miR-99a/let-7c/miR-125b miRNA cluster are of potential interest as non–invasive biomarkers to predict therapeutic response in DLBCL patients. Ongoing experiments in a wider cohort of patients are aimed to confirm these results.

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MEMBRANE AND/OR CYTOPLASMIC CD30 EXPRESSION IN LYMPHOMAS: DOES IT MATTER FOR RESPONSE?

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There is a lack of evidence that brentuximab vedotin (BV) activity is correlated with CD30 expression and it may not be rational to use this marker for strict enrolment purposes even if there is often a cut off expression of ≥10% in clinical trials. Finding a correlation between CD30 expression at baseline and the clinical outcome not only will allow stratifying patients before treatment but also, if necessary, shifting earlier the patient to another therapy. We conducted a retrospective post-hoc analysis on the 15 relapsed/refractory primary mediastinal large B-cell lymphoma (PMLBCL) patients, the majority of which has not responded to BV treatment (EudraCT number 2012-000735-27) with the

aim to compare their CD30 expression pattern (membranous and/or cytoplasmic) with the one of a matched classical Hodgkin lymphoma (cHL) cohort (n= 32) treated with BV at our Institute. Two expert hematopathologists reviewed the samples independently. Among PMLBCL 2 patients had a short partial response (PR) and 1 had a stable disease (SD). The 12 patients who had progression of disease showed a CD30 cytoplasmatic expression while 1 PR and 1 SD others have a membranous one. The cHL cohort was characterized by cytoplasmic positivity in 5/32 patients and membranous/cytoplasmic in 27/32 cases respectively. No difference in CD30 immunohistochemical expression pattern between responder patients and non-responder ones was observed in cHL group. From our observations the prevalent cytoplasmic localization of CD30 in the neoplastic cells seems to be associated with no response in PMLBCL, although the cases are few. Moreover a bias in the analysis could occur since it is not possible to assess if the considered biopsy was performed just before BV therapy as study protocol required assuming CD30 expression from the most recent post-diagnostic biopsy report of relapsed/refractory diseases. Given these limitations, the potential that BV activity may not be only related to CD30 expression on cells requires additional investigation and likely multidisciplinary efforts in order to elucidate its mechanism and identify eligible patients more effectively. In this light, it is possible that immunohistochemistry may not be the only tool to select patients for treatment or that other parameters (such as CD30 prevalent distribution on the neoplastic cells) could play a role in the response. Prospective studies to investigate biologic resistance despite target availability are warranted.

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A PHASE IIIB STUDY WITH SUBCUTANEOUS RITUXIMAB ADMINISTERED DURING INDUCTION PHASE OR MAINTENANCE IN PREVIOUSLY UNTREATED PATIENTS WITH CD20+ DIFFUSE LARGE B CELL LYMPHOMA OR FOLLICULAR LYMPHOMA (MABRELLA): AN INTERIM SUBSET ANALYSIS FROM ITALIAN PATIENTS

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The subcutaneous (SC) administration of Rituximab (RTX) may be beneficial in terms of convenience and tolerability, with potentially fewer and less severe infusion/injection-related reactions (IIRRs) compared to the intravenous (IV) form. The aims were to evaluate the safety, efficacy and PK of SC rituximab in patients with diffuse large B cell lymphoma (DLBCL) or follicular lymphoma (FL). Incidence of administration-associated reactions (AARs), defined as all IIRRs, injection-site reactions, administration site conditions and associated symptoms occurring within 24hr of administration, was the primary study endpoint. The study included adult patients with CD20+ DLBCL or FL who had already received at least one full dose of IV RTX 375mg/m^2 during induction or maintenance phase. Patients on induction received ≥4 cycles of RTX SC 1400mg plus standard chemotherapy and FL patients on maintenance received ≥6 cycles of RTX SC, respectively. At the time of this interim analysis, 126 patients (73 DLBCL and 53 FL, median age 60.5 years, 56.3% of males) were evaluable, 122 completed the induction phase and 44 FL patients entered the maintenance phase. AARs were reported in 8 patients (6.3%), 4 in each group; all were of grade 1 (NCI CTCAE v4.0) and resolved with no dose delay or discontinuation. Treatment-related serious adverse events occurred in 11 patients (8.7%). Two patients (both with DLBCL) had fatal events (Klebsiella infection and septic shock). Neutropenia (45 patient, 35.7%) was the most common grade ≥3 toxicity. Only 4 patients (2 in each subgroup) discontinued rituximab and 25 (19.8%) had dose reduction/delay due to adverse events. High response rates were observed: 63.8% and 68.1% of DLBCL patients, and 67.9% and 73.6% of FL patients had complete response (CR) and CR+CR unconfirmed, respectively (ITT). EFS, PFS and OS at 9 months were 90.4%, 90.4% and 95.9% in DLBCL patients, and 96.2%, 96.2% and 100% in FL patients, respectively. Despite the wide inter individual variability, the geometric mean plasma trough concentrations (Ctrough; ELISA) of RTX increased from baseline to end of induction (DLBCL 48.7 to 87 µg/ml; FL 35.3 to 102 µg/ml). None of the pre-defined covariates (age, sex and FLIPI/IPI risk score) had a significant effect on RTX Ctrough. Our analysis shows that treatment with SC RTX given to DLBCL and FL patients previously treated with at least one dose of RTX IV was associated with high response rates and low risk of AARs in both groups.

P143

RADIOTHERAPY PLUS RITUXIMAB AS FIRST-LINE REGIMEN FOR LOCALIZED FOLLICULAR LYMPHOMA

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Introduction: Follicular lymphoma (FL) is the most common subtype of indolent non-Hodgkin lymphoma; early-stage disease can be cured in a significant proportion with involved-field radiotherapy (IF-RT) alone, however nearly 50% of the patients will ultimately relapse, the majority with lymphoma recurring in non-irradiated areas. A combined association of RT with chemotherapy could increase treatment efficacy, but early and late toxic effects could be unacceptable. *In vitro* synergistic effect between R and RT has been observed, but clinical data that correlates potential benefit of this association in FL patients are limited. *Methods:* in this multicenter study we retrospectively analyzed a cohort of 41 consecutive, early-stage, newly diagnosed FL patients referring to Hematology Divisions of Siena and Florence University Hospitals from 2007 to 2014 receiving rituximab (R) and IF-RT as first-line treatment. We administered R 375mg/m^2 weekly for a total of 4 courses, before or after IF-RT. Primary outcome was PFS, secondary endpoints were CR rate, OS and safety. *Results:* median age was 63 years (range 23-88). Overall, 35/41 patients (80,5%) were diagnosed with stage IA and 6/41 (19,5%) with stage IIA FL. Beta2 microglobulin was above upper limit in 11/41 patients (26.8%), ECOG performance status was 0-1 in all cases.

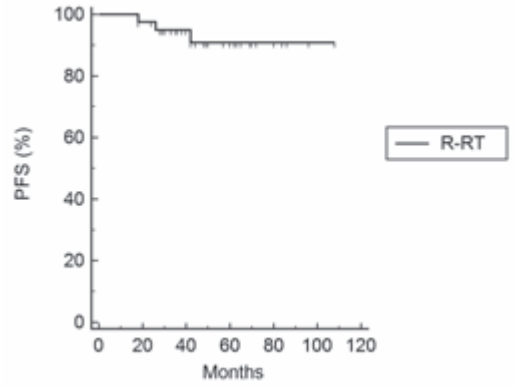

Figure 1.

According to FLIPI2 index, 18/41 patients (44%) presented low-risk, 22/41 patients (54%) presented intermediate risk while the remaining patient was high-risk. IF-RT was administered with a median dose of 24Gy (range 20-44Gy). Bone marrow PCR-BCL2 was positive in 3/41 patients (7%). All patients achieved CR, after a median follow-up of 46 months (range 18-108), all patients remain alive, with only 3 patients relapsing after 18, 26 and 42 months, respectively. Estimated 5-year PFS is 90%, median PFS not reached (Figure 1). Interestingly, 2 patients experienced controlateral relapse in the irradiated area. No hematological toxicity was reported after R administration and infusional reaction were mild. After RT, only transient grade 1-2 mucositis and skin rash

were observed. *Conclusions:* we suggest R in association with IF-RT could represent a suitable first-line treatment option for early-stage FL patients, with higher efficacy and no additional toxicity compared to published data about RT alone. These promising results need to be confirmed in prospective studies, but could pay the way towards an increasing chance of disease eradication.

P144

STUDY OF GENE POLYMORPHISMS AS PREDICTORS OF TREATMENT EFFICACY AND TOXICITY IN PATIENTS WITH INDOLENT NON-HODGKIN LYMPHOMAS RECEIVING BENDAMUSTINE AND RITUXIMAB

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Introduction: indolent non-Hodgkin lymphomas (iNHL) and mantle-cell lymphoma (MCL) have a heterogeneous behavior, impacted by biological and clinical parameters. Bendamustine is widely used in association with rituximab to treat iNHL and MCL. The variability in treatment efficacy and toxicity could be related to genetic factors of the host, such as germline single nucleotide polymorphisms (SNPs) in genes that affect pharmacodynamics and the components of microenvironment. Genetic variants in immune and inflammatory response genes (such as the ones coding for IL-10 and IL-6) and in angiogenic factors (such as VEGF) could affect clinical outcome and side effects. *Methods:* we would like to demonstrate a correlation between SNPs and treatment outcome in iNHL and MCL patients receiving bendamustine and rituximab. We have investigated some SNPs that have already been associated with treatment efficacy and toxicity. All samples were genotyped for the IL-2 (rs2069762), IL-10 (rs1800890, rs10494879), VEGFA (rs3025039), IL-8 (rs4073), CFH (rs1065489) and MTHFR (rs1801131) SNPs by allelic discrimination assays using TaqMan SNP Genotyping Assays (Applied Biosystem) containing primers forward and reverse and allele specific MGB (Minor Groove Binder) probes. SNPs assays were executed on a Rotor Gene 3000 platform system (Corbette, Explera) and the analysis of genotyping were performed using the Rotor Gene Software. *Results:* we have enrolled 54 patients and we report a pivotal analysis of the first 30 iNHL and MCL patients with a follow-up of at least 6 months that received rituximab 375mg/m^2 and bendamustine 90mg/m^2 every 28 days both as first-line treatment (24/30) and as ≥2nd line regimen (6/30). Overall response rate was 100% (CR rate 80%). Treatment toxicity included grade 3-4 neutropenia (12/30 patients), infections (9/30 patients; 1/9 grade ≥3), skin rash (13/30 patients; 1/13 grade ≥3). SNPs in IL-2, IL-8, MTHFR were observed in 12, 12 and 15 patients, respectively; the other investigated genes were wild type for all patients. We did not report any correlation between SNP and CR rate. However, we observed an association between SNP in IL-2 (rs2069762) and skin rash (p=0.03). *Conclusions:* we confirm treatment efficacy and manageable toxicity of rituximab and bendamustine for iNHL and MCL patients. Preliminary results of our study suggest a possible role for cytokine SNPs in bendamustine-related toxicity, that needs to be confirmed in a larger cohort.

P145

THE BERLIN-FRANKFURT-MÜNSTER PROTOCOL FOR THE UPFRONT TREATMENT OF AGGRESSIVE LYMPHOMAS: THE BOLOGNA EXPERIENCE

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Burkitt's lymphoma (BL) and Diffuse Large B-Cell Lymphoma Double Hit (DLBCL-DH) are highly aggressive B-cell lymphomas: due to their fast growing rate, they often represent a clinical emergency. Intensive treatment approaches are required although a univocal standard of care still does not exist. From 2004 to 2016, 51 HIV-negative patients affected by BL (39) or DLBCL-DH (12) received an intensive treatment according to the Berlin-Frankfurt-Münster (BFM) protocol at our Institute. Treatment plan consisted of initial cytoreduction followed by 3 blocks, A (ifosfamide, vincristine, methotrexate, etoposide, cytarabine), B (vincristine, cyclophosphamide, methotrexate, doxorubicin), C (vindesine, methotrexate, etoposide, cytarabine), each repeated twice, every 28 days, with rituximab at day 1 each block. All patients received central nervous system (CNS) prophylaxis with intrathecal methotrexate. Autologous stem cells harvest was done after 4 cycles, with reinfusion (ASCT) at the end of the 6-blocks after BEAM (carmustine, etoposide, cytarabine, melphalan) conditioning. Fourteen patients were females, 37 were males; median age at onset was 41 years. Thirty-nine patients had stage III-IV disease; bulky disease occurred in 25 patients and extranodal involvement in 35, mainly at the gastrointestinal tract (68.6%). CNS involvement was rare (4%). Twelve patients required a preliminary surgical approach, mainly because of bowel occlusion. All but 3 patients received rituximab during treatment; 35 patients completed all the 6 blocks. Stem cell harvest was performed in 34 patients (66.6%) who all received a subsequent ASCT. Treatment withdrawal occurred mainly due to renal toxicities and early patient death in 1 case. Severe cytopenias, all transient and easily manageable, were documented in those who received ASCT. After ASCT, 27 patients (79.4%) achieved a complete response (CR), 24 BL and 3 DLBCL-DH. At a median follow up of 5.5 years, all the patient in CR are alive and disease free; one developed myelodysplasia 1 year after ASCT and 1 acute myelocytic leukemia after 6 years. Four patients died: 2 due to progression of disease, 1 due to a heart failure and 1 due to sepsis after transplant. Intensive treatment according to BFM protocol, with rituximab and ASCT, appears feasible, safe and highly effective in adult patients with BL and DLBCL-DH, as demonstrated by long-term survival rates of patients in continuous CR.

P146

"CIBERSORT"-BASED COMPUTATIONAL ANALYSIS OF TUMOR MICROENVIRONMENT PROVIDES NOVEL PROGNOSTIC GENE PANELS FOR DIFFUSE LARGE B CELL LYMPHOMA

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Background: Diffuse large B cell lymphoma (DLBCL) comprises a group of highly heterogeneous diseases with variable responsiveness to treatment. GEP studies emphasized the role of cell-of-origin (COO) and stromal signatures for stratifying patient risk. However, the clinical translation of such information remains a challenge. Here, we applied an innovative computational method, namely "CIBERSORT", to determine the composition of DLBCL microenvironment and its relationship with molecular and clinical traits of the disease. *Methods:* A matrix of 1028 genes was generated to distinguish 17 different immune and non-immune cell types. This matrix was used to run "CIBERSORT" (http://cibersort.stanford.edu/) on 3 publicly available GEP datasets (GSE10846, GSE19246 and GSE34171; 604 DLBCL cases) and estimate the relative percentage of the diverse tumor-infiltrating cells. Results were stratified according to either COO or clinical outcome. GSEA and Random Forest analyses were then applied to identify genes linked to specific cytotypes.

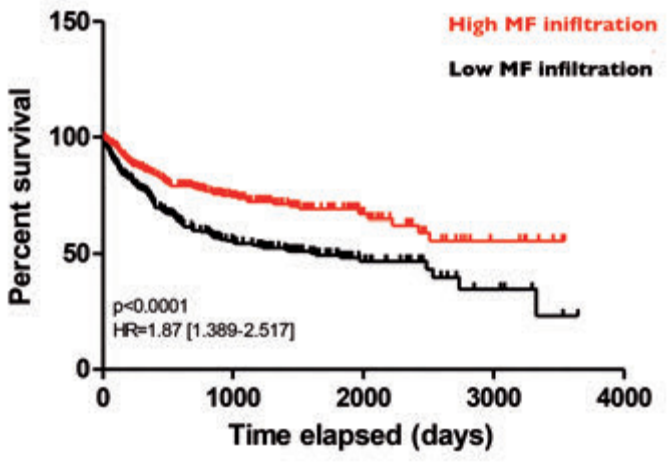

Figure 1.

Molecular biology assays including Real Time PCR and Nanostring technology as well as *in situ* immunostaining were used to measure the expression of selected genes on a cohort of 40 DLBCL cases, and results correlated with the relative clinical data. *Results:* By applying CIBERSORT, we found that plasma cells, NK and dendritic cells showed significant higher proportion in ABC than GCB tumors, whereas CD4 T-cells and DCs prevailed in those with better outcome. Among stromal cells, myofibroblasts showed a significant predominance in tumors with favorable outcome and longer survival (Figure1). Additional bioinformatic analyses produced a panel of 49 genes mostly encoding for extracellular matrix (ECM) proteins showing a significant association with therapeutic outcomes. Finally, preliminary Real-Time PCR data and immunostaining for selected ECM factors supported in silico data, demonstrating an intriguing role immune, stromal and ECM factors in DLBCL biology. *Conclusions:* Estimation of specific tumor-infiltrating cell components revealed unknown aspects of DLBCL microenvironment. Our data suggest that the composition of DLBCL holding peculiar molecular and clinical features also differs in term of immune and stromal infiltration as well as for the expression of specific ECM factors. These findings redesign the prognostic value of reported DLBCL stromal signatures and uncover novel prognostic, and potentially predictive, biomarkers related to tumor microenvironment.

P147

THE ROLE OF 18F-FDG PET/CT IN THE DIAGNOSIS AND FOLLOW-UP OF PRIMARY GASTRIC LYMPHOMA: A MONOCENTER EXPERIENCE

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Introduction: The role of 18F-FDG PET/CT in the diagnosis and follow-up of primary gastric lymphoma is still controversial. In particular, regarding gastric MALT lymphoma staging several studies didn't clearly define the detection rate of PET/CT imaging. The aim of our study is to evaluate the role of 18F-FDG PET/CT in the diagnosis and follow-up of primary gastric lymphoma. *Materials and Methods:*Thirty-two patients with histological confirmed gastric lymphoma (MALT: 19; DLBCL:13) underwent a 18F-FDG PET/CT for initial staging and post therapy evaluation. The PET images were analyzed visually and semi-quantitatively by measuring the maximum standardized uptake value (SUV MAX) and compared with Ann Arbor stage, epidemiological (age, sex), histological /morphological (presence of gastritis, ulcers, H. pylori infection, tumor size, superficial lesions or mass-forming) characteristics. *Results:* From January 2007 to November 2016, in our institution, we analyzed 32 primary gastric lymphoma 19 patients had histological diagnosis of MALT lymphoma, whereas 13 patients received histological diagnosis of DLBCL. At diagnosis, 15 patients with MALT lymphoma had positive PET/CT-mean lesion SUV max of 6.14 (4.0-18.2) at the corresponding gastric lesion, the remaining 4 were not 18F-FDG avid. On the other hand, all 13 patients with DLBCL had positive PET/CT, as expected, with mean lesion SUV max of 17.1 (4-17.1). Eighteen patients with MALT lymphoma underwent a chemotherapy, while only one underwent a H. pylori eradication treatment. All 13 patients with DLBCL underwent a chemotherapy. At post-treatment evaluation, all the patients had histological and radiological complete remission. The overall sensitivity of 18F-FDG PET/CT was 87% (CI 95%: 71-96.5) and specificity 100% (CI 95%: 89.1-100) (p value<0.0001) in our cohort. In MALT lymphoma PET sensitivity was 78% (CI 95%: 54.4-93.5) and specificity 100% (CI 95%: 82.3-100) (p value<0.0001), whereas in DLBCL the sensitivity (CI 95%: 75.3-100) and specificity (CI 95%: 75.3-100) were 100% (p value<0.0001). *Conclusions:* Based on our data, 18F-FDG PET/CT appears to be accurate for initial staging and post treatment follow up in patients with MALT lymphoma and DLBCL. According to our observations, 18F-FDG PET/CT could be used to detect early relapse together with the histological evaluation. Our results should be considered as a preliminary study, limited at our cohort of 32 patients, that will be enlarged with new data.

P148

PREDICTIVE FACTORS FOR INFECTIOUS ADVERSE EVENTS IN PATIENTS WITH B-CELL NON-HODGKIN LYMPHOMA (B-NHL) TREATED WITH BENDAMUSTINE-RITUXIMAB (R) RESULTS OF A RETROSPECTIVE ANALYSIS

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Background: The combination of bendamustine (B) and rituximab (R) is an effective and well tolerated therapy for B-NHL. However, previous reports have shown high incidence of lymphopenia and secondary infections in pts treated with BR. We performed a retrospective analysis at our center with the aim of determining the incidence of the infectious adverse events (AEs) and potential predictive factors. *Methods:* We collected data from 65 pts with B-NHL who received at least 2 cycles of BR between 2010 and 2016 at our center. The AEs including neutropenia (N), neutropenic fever (NF), lymphopenia, infections episodes and the occurrence of second tumors, were recorded according to the CTCAE score. We compared the risk factors of pts who developed infections and those who did not. Univariate analysis was used to evaluate the potential risk factors. *Results:* The median age at the first cycle was 66 yrs (36-89), 33 pts (50%) were ≥65 yrs, 41% were male, 82% had advanced disease and 60% had bone marrow involvement. Thirty (46%) pts had FL, 17 (26%) MCL, 11 (17%) MZL, 5 (7%) DLBCL and 4% other indolent lymphomas. Thirty two pts (49%) received BR as first line treatment, 51% as second line and above. Six cycles of B was administered either at the dose of 70 or 90 mg/mq iv on days 1, 2 and R was administered at a dose of 375 mg/mq, on day 1. The mean number of cycles was 5 (2-6), 13 pts (20%) discontinued due to toxicity: 8/13 for non-hematologic AEs. Primary or secondary G-CSF prophylaxis was given to 25 pts (38%), while the prophylaxis against PJP was given to all pts. Twenty two pts (34%) had at least one infection. Bacterial pneumonia was identified in 6/22 pts, VZV infection in 4/22, CMV reactivation in 2/22 and other infections in 10 pts. At univariate analysis, the infectious AEs were associated only with lymphopenia during the second cycle (p=0.043) and with N during the II, III and IV cycle (p=0.026; p=0.003, p=0.018, respectively). Other AEs were: grade 3/4 N (49%), NF (3%), grade 3/4 lymphopenia (80%). We reported also a 5% incidence of second tumors after treatment. *Conclusions:* In our analysis, BR confirms a safety profile similar to that reported in previous studies. According to our results, an early lymphopenia and neutropenia are predictive factors for infections AEs and for premature treatment discontinuation. These data raise the question on the role of antibacterial, antiviral and primary G-CSF prophylaxis in all pts treated with BR.

P149

EFFICACY, SAFETY AND COST ANALYSIS OF SUBCUTANEOUS VS INTRAVENOUS RITUXIMAB IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH RCHOP

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Introduction: Rituximab (R) in combination with cyclophosphamide, doxorubicin and prednisone (R-CHOP) is the standard of care for patients (pts) with diffuse large B-cell lymphoma (DLBCL). A subcutaneous (sc) formulation that provides a fixed dose of R is being tested in a number of studies. Results indicate that the pharmacokinetics is not inferior and the response rates comparable to those obtained with the intravenous (iv) formulation. In August 2014, the Italian Medicine Agency (AIFA) approved the sc formulation for follicular lymphoma and DLBCL. We performed a retrospective analysis at our Centre in pts with DLBCL treated with RCHOP. The aim was to evaluate the costs of the 2 different formulations of R (sc *vs* iv) combined with CHOP and the efficacy in terms of complete response (CR) rates and toxicity. *Methods:* We collected data from 71 consecutive pts with untreated DLBCL who received 6 cycles of RCHOP plus 2 doses of R between January 2014 and January 2016; 35 pts received iv R (375mg/mq) and 36 sc R (1400 mg) from May 2015. We compared the direct costs of the 2 formulations of R, the rate of CR and of adverse events (AEs) of the two

subgroups of patients. Univariate analysis was used to evaluate efficacy and toxicity. *Results:* The clinical characteristics were well balanced between the iv and sc RCHOP groups: mean age 61 years in both groups, with a mean BSA of 1.8 (1.4-2.2); IPI score ≥3, 20% *vs* 30%; Ann Arbor stage III-IV, 62% *vs* 69%. There was no significant difference in terms of efficacy: the CR rates were 30/35 (85%) and 29/36 (83%), p=0.177, respectively. Grade ≥3 AEs (45% *vs* 47%) were almost all hematologic (90% in both groups) and the most common AE was grade 3/4 neutropenia in both groups. The cost of the treatment was 472.227€ for the iv R group and 449.870€ for sc R group; with a decrease of 4.73% only of the direct costs of R. In addition, the calculated infusion time for the sc RCHOP was 135 min compared to 240 min for the iv RCHOP; this translated into a 44% chair time reduction. *Conclusions:* In our analysis, the use of a sc formulation of R allowed a gain of 22.357€ in terms of only direct costs compared to the schedule with iv R, with comparable response rates and similar safety profile. Given the shorter delivery time, the sc formulation could also improve pts' comfort and reduce the burden on health care resources. Finally, the reduction in required chair time allows for a greater number of pts to be treated daily.

P150

VALIDATION OF CNS-IPI AND CORRELATION WITH CELL OF ORIGIN PROFILE IN A RETROSPECTIVE SERIES OF DLBCL PATIENTS TREATED WITH R-CHOP IN A SINGLE INSTITUTION

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Central nervous system (CNS) recurrence of diffuse large B-cell lymphoma (DLBCL) is a dramatic event, occurring in roughly 5% of patients treated with standard rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP). The CNS-International Prognostic Index (CNS-IPI) (Schmitz et al, JCO 2016) is a recent, valid and highly reproducible score to estimate the risk of CNS recurrence in DLBCL treated with R-CHOP. The aims of this restrospective analysis were to investigate the use of CSN-IPI in a cohort of DLBCL patients treated with R-CHOP in a single institution and correlate CNS recurrence with cell of origin (COO) profile assessed with immunohistochemistry (IHC) according to Hans algorithm. CNS-IPI factors (age, lactate dehydrogenase (LDH) serum levels, stage, ECOG performance status, number of extranodal sites, involvement of kidney and/or adrenal gland) were recorded and patients classified into three groups of CNS-relapse risk (low, intermediate and high) according to CNS-IPI. We calculated the cumulative incidence of CNS-relapse in the whole cohort and stratified in the three risk group with Gray' test.

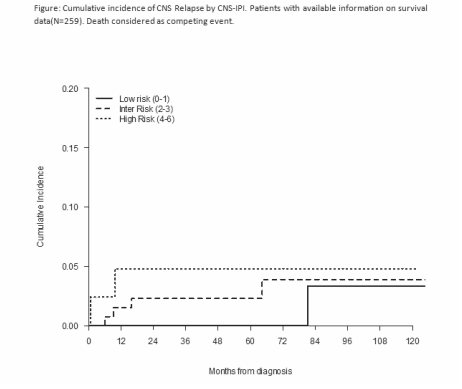

Figure 1.

A total of 259 patients with DLBCL at diagnosis, treated with R-CHOP between 2003 and 2013, were included in the analysis. Median age was 65 years (range 55-73), 176 (68%) patients had advanced stage (III-IV) disease, 124 (48%) high LDH serum level, 56 (22%) ECOG PS >1. By COO, 85 (33%) cases were germinal center (GCB), 107 (41%) were non-GCB and 47 (18%) were not evaluable. 68 (26%) patients received intrathecal (IT) methotrexate for CNS prophylaxis per local practice. No patients received intravenous methotrexate. According to CNS-IPI, 81 (31%) patients were classified at low risk, 136 (53%) at intermediate and 42(16%) at high. In the whole population, 8 CNS relapse were observed; 4 (50%) of 8 cases received CNS-IT- prophylaxis and 6 (75%) were non-GCB at COO. 3-years Cumulative incidence of CNS relapse (CI) was 2% (95%CI: 0.3% to 3.7%) in the whole cohort; CIs stratified according to CNS-IPI were: 0% in the low risk group, 2.3% (95%CI: 0% to 4.8%) in the intermediate risk and 4.8% (95%CI: 0% to 11.3%) in the high risk group. With a median follow up of 60 months, in the whole group, 5-years Progression Free Survival (5-ys PFS) was 90%, 5-ys Overall Survival (5-ys OS) was 91%. In conclusion, CNS-IPI represent a valid score to estimate risk of CNS relapse, that remains an important unmet clinical issue for treatment of DLBCL patients.

P151

A NOVEL IMMUNOHISTOCHEMISTRY SCORE BASED ON MYC, BCL2 AND BCL6 EXPRESSION ALLOWS TO IDENTIFY DLBCL SUBSETS AT DIFFERENT PROGNOSIS: RETROSPECTIVE ANALYSIS OF DE NOVO DLBCL TREATED WITH RITUXIMAB-CHOP

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Whereas the poor prognosis of Double Hit Lymphoma (DHL) is well known, the prognostic role of MYC, BCL2 and BCL6 expression, assessed by Immunohistochemistry (IHC) is still contradictory. The aim of the present study was to assess the prognostic role of MYC, BCL2, and BCL6 expression in a retrospective cohort of de-novo DLBCL, treated consecutively with R-CHOP between January 2003 and December 2013. BCL2, BCL6 and MYC expression were evaluated by IHC and by Tissue Micro Arrays (TMA) technique; cases were considered positive for MYC, BCL2 or BCL6 expression by IHC if >40%, >40% or >25% of cells stained positive, respectively. FISH analysis for MYC and BCL2 rearrangements were also performed with dual color break apart probes on TMA. Progression Free Survival (PFS) and Overall Survival (OS) were estimated with Kaplan-Meier method and compared between groups with the Cox model. In a pilot series, Cox multivariate regression model adjusted for IPI and age, MYC overexpression, BCL2 positivity and BCL6 negativity showed prognostic relevance; established that these 3 variables contributed with different risk, an IHC sum additive score of 0-5 was calculated assigning an individual risk of 2 points for MYC or BCL2 positivity and 1 point for BCL6 negativity.

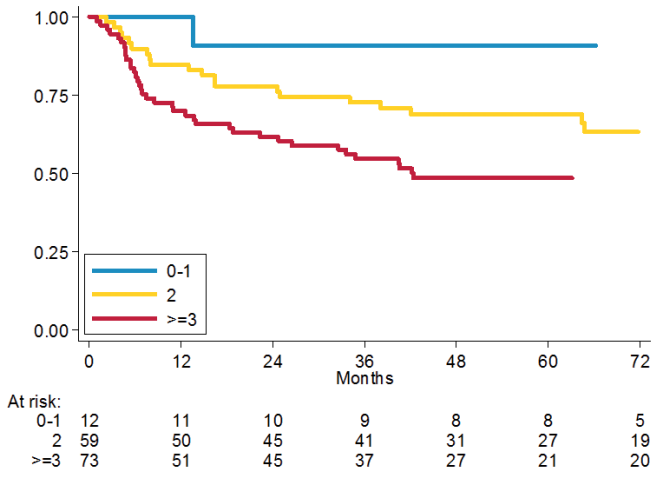

Figure 1.

Patients were stratified in 3 risk groups; Low (0-1 point) (with or without BCL6), Intermediate (2= MYC or BCL2 single overexpression) and High risk (≥3) (MYC+/BCL6-, BCL2+/BCL6-, MYC+/BCL2+, MYC+/BCL2+/ BCL6-). Of a total of 297 DLBCL included into the study, 265 were evaluable for survival analysis; median age was 65 years (range 20-90); 152 were analysed by IHC and 100 of 152 by both IHC and FISH. No bias selections were observed in the 2 groups. Among 100 patients investigated by FISH we recorded 8 DHL (8%); with a median follow up of 60 months PFS in DLBCL and DHL was 66% and 25% respectively [HR 3.05 (95% CI: 1.26- 7.36, p 0.013)] and 5 ys OS was 80% and 25% respectively [HR 3.63 (95% CI:1.48- 8.91 p 0.005)]. Excluding those 8 DHL, the remaining 144 patients with complete IHC data were: low risk 12 (8%), intermediate risk 59 (41%) and high risk 73 (51%). 5y-PFS rates were 91% *vs* 68% *vs* 48% (p=0.014) respectively (HR per unit increase 1.63 (95% CI: 1.14 -2.55) p 0.032), (Fig1). Our data showed that in a large cohort of DLBCL treated with standard R-CHOP, excluding DHL, our simple IHC score based on MYC, BCL2 and BCL6 expression had a prognostic value and was able to identify three groups at different outcome.

Non Hodgkin Lymphomas 2

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EFFICACY AND SAFETY OF BENDAMUSTINE IN ASSOCIATION WITH RITUXIMAB AS FIRST-LINE TREATMENT FOR PATIENTS WITH INDOLENT NON-HODGKIN LYMPHOMA OR MANTLE CELL LYMPHOMA

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Introduction: Bendamustine in association with rituximab (B-R) showed high efficacy and favourable toxicity for treatment of relapsed/refractory indolent non-Hodgkin lymphoma (iNHL) and mantle-cell lymphoma (MCL) not suitable for high-dose therapy. According to these promising results this regimen was successfully investigated as first-line treatment, showing similar efficacy and reduced toxicity compared to R-CHOP in 2 phase III randomized trials. In this study we have evaluated efficacy and feasibility of B-R as first-line treatment in a real life setting. *Methods:* We analyzed 40 consecutive previously untreated patients with iNHL (follicular lymphoma, marginal zone lymphoma, lymphoplasmacytic lymphoma) or MCL not suitable for high-dose therapy diagnosed at our Institution that received B-R from April 2010 to August 2016. The treatment regimen consisted of rituximab 375mg/m^2 intravenously on day 1 and bendamustine 90mg/m^2 on days 2-3 every 28 days for up to 6 cycles, providing that hematological recovery (neutrophil count 1.5x10^9/L, platelet count 100x10^9/L) had occurred. Secondary prophylaxis of febrile neutropenia with filgrastim was administered if grade>2 neutropenia occurred. Response to treatment was assessed after 3-4 cycles and 1 month after last course with CT scan and bone marrow biopsy (if positive at diagnosis). *Results:* Out of 40 patients, 39 achieved a response, with an overall response rate of 97,5%, 30/40 (75%) achieved a complete response (CR). Response rates were similar for all the different histologies. Interestingly, all 6 MCL patients achieved a CR. Grade 3-4 hematological toxicity was reported in 11/40 patients (27,5%), 10/11 cases had grade 3-4 neutropenia. FUO and febrile neutropenia were observed in 10/40 (25%) and 4/40 (10%) patients, respectively. Non-hematological toxicity was mainly represented by skin rash, that occurred in 12/40 patients (30%), 3 out of 12 cases experienced grade 3 skin rash. After a median follow-up of 20,5 months, 2 patients relapsed and 2 patients died, 1 because of second neoplasm and the other because of heart failure. Median PFS and OS were not reached, estimated 4-y PFS was 65%. *Conclusions:* in our study we confirm high long-term efficacy and manageable toxicity of B-R regimen as first-line therapy for iNHL and MCL patients not suitable for high-dose therapy in a real life setting of unselected cases.

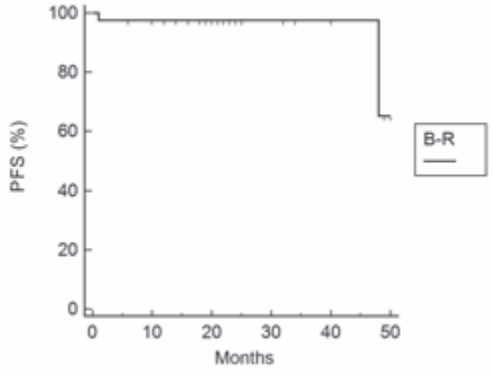

Figure 1.

P153

USE OF LIPEGFILGRASTIM IN CLINICAL PRACTICE FOR THE PROPHYLAXIS OF CHEMOTHERAPY-INDUCED NEUTROPENIA IN LYMPHOMA PATIENTS: INTERIM RESULTS OF A PAN-EUROPEAN NON-INTERVENTIONAL STUDY

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Background: Lipegfilgrastim (Lonquex®) is a long-acting fixed-dose glycopegylated granulocyte colony-stimulating factor administered once per chemotherapy cycle. It has been available in Europe since 2013. It was proven to be non-inferior with regard to duration of severe neutropenia compared with pegfilgrastim in breast cancer patients. However, data in patients with hematological malignancies are limited. *Aims:* We aimed to evaluate the effectiveness of Lipegfilgrastim in the cycle following the first lipegfilgrastim-supported treatment cycle in lymphoma patients. *Methods:* This is a prospective observational cohort study. Patients with different tumor types treated with cytotoxic chemotherapy (CT) who received lipegfilgrastim in primary prophylaxis (PP) or secondary prophylaxis (SP) are being included in this study. CT dose modifications and neutropenia-related events are recorded and analyzed. Evaluation of effectiveness in the cycle following the first lipegfilgrastim-supported CT cycle in a lymphoma subpopulation is presented here. *Results:* At the time of the interim analysis (Dec. 2016), 249 patients diagnosed with lymphoma have been included. Mean age ± standard deviation of lymphoma patients was 61.6±15.6 years and 56.6% were male. For the majority of patients (81.1%) intended use of lipegfilgrastim was in PP. Exposure to lipegfilgrastim has been documented for 228 patients with an average of 4.76 cycles per patient. Data on CT dose modifications and neutropenic events following the first lipegfilgrastim-supported cycle were available for 144 and 167 patients, respectively. CT dose was never omitted. CT dose delays were observed in 8.0% (PP) and 18.8% (SP) of patients and CT dose reductions in 4.5% (PP) and 12.5% (SP) of patients. In the first lipegfilgrastim-supported cycle, febrile neutropenia was recorded in 4.5% (PP) and 3.0% (SP) of patients; severe neutropenia was recorded in 7.5% (PP) and 9.1% (SP) of patients. Throughout the treatment, 22 (9.6%) patients exposed to lipegfilgrastim reported at least 1 adverse drug reaction (ADR). The most common ADRs were myalgia and musculoskeletal pain. Serious ADRs were reported by 11 (4.8%) patients. *Conclusions:* Lipegfilgrastim is effective and well tolerated in the real-world setting in lymphoma patients, administered either in PP or SP. The results suggest that lipegfilgrastim administered in PP might give better outcomes in terms of dose delays and dose reductions than when administered in SP.

P154

MANAGEMENT, ECONOMIC AND SOCIAL IMPACT OF SUB-CUTANEOUS RITUXIMAB ADMINISTRATION IN DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) AND FOLLICULAR LYMPHOMA (FL)

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Introduction: DLBCL and FL represent frequent hematologic malignancies generally treated and followed in Hematological Day Hospital (DH) which therapeutic programs are time consuming and costing and may affect the Quality of Life (QoL) because of the prolonged stay in DH. *Aim:* To evaluate, in these patients the economic and social impact of subcutaneous Rituximab (R) formulation. *Methods:* From March 2016 to December 2016, we submitted a questionnaire of satisfaction to 45 pts receiving R and 45 caregivers; moreover, we investigated the advantages of subcutaneous R administration compared to intravenous formulation by evaluating, during one week, several parameters and quantifying time consumption and quality of life (QoL). Furthermore, we evaluated its role in the optimization of DH management in terms of waste of time, professionals and economic expenses and in improving patients' safety and satisfaction. *Results:* Among the 45 patients, 55% were affected by DLBCL and 45% by FL, 64% were males and 36% females; as for age 68% were over 60 years. The questionnaire examined patient's emotions and perceptions during R administration (anxiety, fear and pain), time required for infusion and interference with daily activities. Overall, 98% of interviewed patients preferred subcutaneous administration because less scared by this formulation and because of the reduced waste of time. Among the 45 interviewed caregivers 68% were workers. They considered advantageous the subcutaneous formulation of R because of the reduced waste of time. Comparing patients treated with subcutaneous R formulation to those who received the IV formulation:_ 15% *vs* 47% (P=0.003) considered time of infusion annoying and 13% *vs* 46% (P= 0.001) were conditioned in daily activities. Infusion of subcutaneous R formulation was perceived as long as expected or even shorter by 80% of patients *versus* 46% of those who received intravenous formulation (P=0.002). Other results are reported in table 1. *Conclusions:* Our investigation shows that patients receiving subcutaneous R formulation and their caregivers are more satisfied of this formulation that in addition produces an important reduction of costs equal to 274.486€/pts treated by sc R formulation. Considering that in the year 2016 we have administered 240 sc doses of R, we saved 65,8761.64 € (the yearly salary of 2 nurses).

Table 1. Comparison of time and costs by iv and sc Rituximab.

TIME NEEDED	iv Rituximab	sc Rituximab	Δ (= IV-SC)
• Total time needed for 8 R administration (1 iv +7 sc)	46.2h	28.7h	+38% (17.5h)
• Total time needed to manage 1 pts/day	144 min	111 min	+23% (33 min)
• Average time to prepare 1 dose of R /day	40 min	19min	+53% (21 min)
YEARLY COSTS 2016	# 470 doses	# 240 doses	
• Rituximab Costs/Pts	1,750	1,593.73	+156.27 € (IV-SC)
• Nurse*	0.342€/min*144= 49.248 €/pts	0.342€/min*111= 37.962€/pts	+11.286 €/Pts
• Technician**	0,33 €min*40min =13.2 €/pts	0,33 €min*19min =6.27 €/pts	+6.93 €/pts
• DH Armchair***	100€/pts	Not Required	+100 €
TOTAL SAVING/Pts			274.486 €/pts

Basal assumption for the analyses: Patients Mean BSA (Body Surface Area) : 1.86 m² (total dose of IV Rituximab required 700mg); SC formulation cost/syringe 1.593,73 €; IV formulation cost 1.750 € (=2,5 €/mg*700 mg) . * Nurse yearly cost 36,909 € =3.075.75 € mos = 20.505€/hr (considering a total of 150 Monthly Hours at work)= 0.342 €/min. **Technician yearly costs 37,206 € =3,017 € mos = 20.114€/hr (considering a total of 150 Monthly Hours at work)= 0.33 €/min. ***DH Armchair use cost 100 €/day (data provided by Program and Management Control Area).

P155

RITUXIMAB-BENDAMUSTINE-DEXAMETHASONE (RD-BENDA) FOLLOWED BY RITUXIMAB CONSOLIDATION AND LENALIDOMIDE MAINTENANCE FOR FRAIL ELDERLY PATIENTS WITH NEWLY AGGRESSIVE B-NON HODGKIN LYMPHOMA

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Frail elderly patients (pts) with aggressive B non-Hodgkin Lymphoma (a-B-NHL) in most cases show comorbidities precluding the use of antracycline-based standard regimens. The safety and efficacy of bendamustine, rituximab plus dexamethasone (RD-Benda) regimen were prospectively investigated in 14 elderly frail with newly diagnosed a-B-NHL. Fourteen (4 female, 10 male) frail elderly pts (median age: 79 years; range 68-86 years) with a-B-NHL (11 Diffuse Large B-Cell Lymphoma, 1 Burkitt NHL, 1 Burkitt-like NHL and 1 Mantle cell lymphoma) were enrolled in a phase II study with bendamustine 70 mg/mq i.v. on days 1 and 2, rituximab 375 mg/mq i.v. on day 1 and oral dexamethasone 20 mg total dose on days 1-4 for four cycles. Pts who showed complete (CR) or partial response (PR) after the fourth induction cycle of RD-BENDA started a consolidation course with four weekly doses of rituximab (375 mg/mq i.v.) followed, in the case of persistence of CR or PR, by a maintenance treatment with monthly courses of lenalidomide (10 mg/mq, days 1-21). Pts with progressive disease after RD-BENDA started maintenance therapy with monthly courses of full dose lenalidomide. After a median follow-up of 6 months (range 2-18), the overall response rate was 81%, with CR and PR of 63% (n=7) and 18% (n=2), respectively. Two pts died due to multiple organ failure and disease progression after 1 and 8 months from diagnosis, respectively. In our frail and elderly patient cohort, the sequential treatment strategy was well-tolerated. After R-BENDA cycles, grade II infectious disease was observed in 2/11 pts (18%) and DNA-CMV reactivation was detected in other 2 additional pts (18%). However, 2 out of five pts who started maintenance lenalidomide treatment discontinued therapy for renal and hematological grade 3 toxicity. At the time of analysis, the estimated median 18-month progression free survival and overall survival were 75 and 66%, respectively. Our preliminary data show that

sequential treatment with RD-BENDA followed by four weekly doses of rituximab and finally by lenalidomide maintenance is a feasible and safe therapy option in frail elderly a-B-NHL pts, but needs to be assessed in a larger subsequent trial.

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PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA: A 10 YEARS MONOCENTER EXPERIENCE IN CLINICAL DAILY PRACTICE

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Introduction: primary central nervous system lymphoma (PCNSL) is a rare form of aggressive extranodal non-Hodgkin lymphoma. High-dose methotrexate (HD-MTX) is the backbone of systemic treatment; additional advantage was reported in IELSG32 trial by adding HD-cytarabine, rituximab and thiotepa, that we can consider as the best available induction. Optimal consolidation is not defined; whole brain radiotherapy (WBRT) was associated with high remission rates but significant neurotoxicity, while autologous stem-cell transplantation (ASCT) seems promising in younger fit patients. We report treatment strategies and outcomes in our unselected PCNSL patients. *Methods:* we retrospectively analyzed 20 consecutive PCNSL patients treated at our Institution from 2005 to 2016. Treatment included HD-MTX with cytarabine, or this combination plus rituximab or plus rituximab and thiotepa; elderly-unfit patients not suitable for HD-cytarabine received HD-MTX and temozolomide. Induction was followed by WBRT or ASCT as consolidation. *Results:* median age was 61,5 years, ECOG PS was ≥2 in 13/20 patients (65%), IELSG score was low, intermediate and high in 4, 14 and 2 patients. Five out of 20 (25%) had early treatment discontinuation after 1 cycle because of disease progression or toxicity and were analyzed separately in survival curves. Fifteen patients received at least 2 courses, 10/15 were responders (66,6%), 5/15 (33,3%) achieved a CR. The 2 patients >70 years receiving HD-MTX and temozolomide had PD. Grade 3-4 hematological toxicity was reported in all cases treated with cytarabine. All 10 responders received WBRT (7 patients) or ASCT (3 patients) as consolidation, 3 cases receiving WBRT and 1 receiving ASCT improved response from PR to CR. Neurotoxicity was reported in 5 patients (4/5 received WBRT). Median PFS and OS for patients receiving at least 2 courses were improved compared to early discontinuation group (12 *vs* 2 months, p=0.03; 10 *vs* 4 months, p=0.01). In the first group estimated 5-y PFS and OS were 35% and 38%. *Conclusions:* in the real life it is often hard to offer an adequate treatment for PCNSL because of age, diagnostic delays and poor PS. Optimal induction should include HD-MTX, cytarabine, rituximab and thiotepa in younger fit patients; the group receiving at least 2 courses could have better prognosis. Consolidation is useful, WBRT and ASCT are both effective, with a warning for WBRT about neurotoxicity, while ASCT is precluded for elderly-unfit patients.

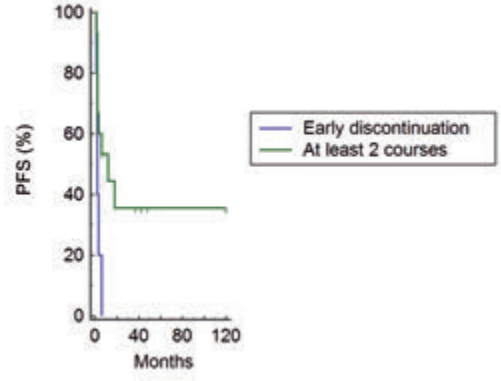

Figure 1.

P157

THE ROLE OF VITAMIN D AS A PROGNOSTIC FACTOR IN DIFFUSE LARGE B CELL LYMPHOMA: A MONOCENTRIC STUDY FROM HEMATOLOGY UNIT OF REGGIO EMILIA

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Vitamin D (VitD) insufficiency is common in western countries. Recent data suggest that VitD insufficiency is related to poor prognosis in Diffuse large B-cell lymphomas (DLBCL) and Follicular lymphoma (FL). We tested the hypothesis that VitD levels are predictive of progression free survival (PFS) and overall survival (OS) in a retrospective cohort of newly diagnosed patients with DLBCL treated in the Hematology unit of Reggio Emilia between 2013 and 2016. Results. We identified 50 patients (pts) who were eligible for this study. Median age was 70 years (24-93); 23 were males, stage III-IV was found in 38 pts, LDH was elevated in 27 cases (55%), IPI was high (3-5) in 27 cases (55%). R-CHOP like regimen was the most commonly employed (34; 70%) and was used at reduced doses in 7 cases. Response to therapy was complete in 74%. Baseline median serum VitD levels were 16.1ng/ml (range <4 to 38.8ng/ml); 7 pts had normal VitD levels, 26 had a mild to moderate reduction (24 to 10ng/ml), and 16 pts (32%) had severe reduction of VitD levels (<10ng/ml). Serum VitD levels did not differ between age groups (<60 *vs* >= 60 years), stage (I-II *vs* III-IV), IPI risk groups (0-2 *vs* 3-5), and Performance Status (PS; 0-1 *vs* 2-4). Median follow-up was 20 months (3 to 38); 3 years OS and 3 years PFS were 69% (95% IC 51-82%) and 66%(95%IC 47-80%), respectively. Prognostic role of serum VitD levels and of the main clinical parameters were tested in univariate analysis for both PFS and OS. For the analysis of VitD we evaluated two cut-offs: the best study cutoff defined at 15ng/ml and a more conventional cut-off at 10ng/ml. Both cut offs were associated with a different risk of PFS; HR for low serum VitD levels was 3.52 (1.15–10-8) and 6.45 (1.43–29.2) for PFS using the 10 and 15 cut-offs, respectively. Regarding OS only the 15ng/ml cut off was associated with a significantly increased risk of death (HR 5.76 (1.26–26.3), along with elevated LDH, poor PS, high risk IPI and low serum albumin. *Conclusions:* Serum VitD insufficiency is frequent among pts with DLBCL and was found severe in a third of cases. In univariate analysis of PFS and OS, the poor prognosis of pts with very low levels of serum VitD is confirmed in this setting of pts. Additional analysis to extend our study to more patients and to other lymphoma subtypes and to test the efficacy of adequate correction of baseline VitD levels are planned.

P158

COMPUTER-ASSISTED QUANTIFICATION OF TUMOR CELLS AND T CELLS IN DIFFUSE LARGE B CELL LYMPHOMA

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Diffuse large B cell lymphoma (DLBCL) is the most common form of non-Hodgkin lymphoma, accounting for about 31% of all non-Hodgkin's lymphoma (NHL) cases in western countries and 37% of B cell cancers worldwide. DLBCL presents a high clinical and biological heterogeneity, supporting the notion that most of these lymphomas arise from germinal center B-cells at different stages of differentiation, in which recurrent genetic alterations contribute to the molecular pathogenesis of the disease. Advanced high resolution digital scans of pathology slides have allowed the development of computer-based image analysis algorithms that may help pathologists in IHC stains quantification. In this study we evaluated, using a computerized cell quantification method, tumour cells and T cells in 29 patients with Diffuse Large B-cell Lymphoma (DLBCL). CD20 and CD3 count were assessed with the Positive Pixel Count algorithm embedded in the Aperio ImageScope software and reported as percentage of positivity, defined

as the number of positively stained pixels on the total pixels in the image. A statistically significant difference was observed in the percentage of tumor cells in different histological and clinical manifestations. Specifically, a lower tumor cells count was observed in patients with a non germinal center immunophenotype, high LDH, splenomegaly and an IPI ≥3. A statistically significant difference was observed in the number of CD3 in patients with bulky disease and an IPI≥3. Specifically, a lower number of CD3 was observed in bulky disease patients (mean count 28%) compared to non-bulky disease patients (mean count 46%), p value <0.05. A lower number of CD3 was observed in patients with an IPI ≥3 (mean count 32%) compared to IPI <3 (mean count 44%), p value<0.05. A lower number of CD3 was observed in patients with stage 3-4 (mean count 30%) than stage 1-2 (mean count 45%), p value<0.05. An improved understanding of tumor biology and the role of the tumor microenvironment has led to advances in the diagnosis, classification, prognostication of patients with hematologic malignancies, as well as novel treatments. In particular, translational research has provided many promising new approaches to cancer therapy, leading to drugs that target the interaction between the tumor microenvironment and malignant cells.

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CAUSES OF DEATH IN FOLLICULAR LYMPHOMA: RESULTS OF A TEN-YEAR FOLLOW-UP FROM THE MONOCENTRIC RETROSPECTIVE STUDY

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Introduction: Follicular lymphoma (FL) is an incurable disease with frequent relapses and shorter response to further treatments. Although the life expectancy of patients with FL has recently increased, notably since the introduction of rituximab in combination with chemotherapy, little is known regarding the precise causes of patients death. The aim of this study was to evaluate the causes of death in a long term follow-up. *Patients and Methods:* We performed retrospective analysis of patients with grades 1-3a FL diagnosed from January 2000 to December 2004 at our institution, to evaluate long-term follow-up outcomes (>10 years). We considered all patients with this diagnosis regardless to treatment and considering also patients in watch and wait. *Results:* 146 patients were diagnosed and treated at our Institution. The median age at diagnosis was 61 years (range 30-87). Stage I-II in 47 patients, III-IV in 86. FLIPI 0-1 in 40, FLIPI 2 in 48, FLIPI 3 in 40 and FLIPI 4 in 18 patients. According to treatment 98 patients were treated with antracycline containing regimens, 34 with fludarabine containing regimens and only 14 were observed or treated with radiotherapy. Most of the patients 67% received Rituximab: 50% as part of sequential treatment and 16% as part of the initial chemotherapy. The median follow-up was 13,4 years (range 11-15) for living patients and 8 years (range 0,09-15) for dead patients. At the time of this report sixty-five patients (44%) have died, the causes of death were lymphoma progression (54%), second malignant neoplasms (25%), other disease (18%), 1 car accident and 1 unknown. At 10 years, overall survival rate was 71%. Low FLIPI score, the use of Rituximab and obtainment of complete remission after induction were associated with superior overall survival in univariate analysis. Multivariate analysis using the above factors demonstrated FLIPI 0-1 and achievement of complete remission as the only factors associated with better outcome. Exactly the same results were observed if we considered the cause specific mortality. In conclusion this study confirms that the most common causes of death in patients affected by FL are lymphoma progression, complications related to therapy and second malignancies. Despite advances in therapy and supportive care of patients with FL, many patients still die of this disease or of sequelae related to its treatment. *Aknowledgments:* This study was supported by the Legato Zottola Donation and AIL Pistoia.

P160

ADVANCED MRI: A VALUABLE ALTERNATIVE TO FLUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY (FDG-PET/CT) FOR EVALUATE RESIDUAL DISEASE AT THE END OF THE THERAPY IN LYMPHOMA. A PROSPECTIVE MONOCENTRIC STUDY

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Purpose: To test the diagnostic performance of advanced MRI (Diffusion Imaging, DwI, and Perfusion Imaging, PwI) in the evaluation of residual masses in Hodgkin (HL) and non-Hodgkin (NHL) lymphoma patients (pts), compared to computed tomography (CT) and PET/CT. We aimed to investigate if MRI can decrease the false positive or false negative rate and if it can be an alternative to the PET/CT. *Methods:* We enrolled prospectively consecutive pts with diagnosis of HL and follicular (FL) or diffuse large B-cell lymphoma (DLBCL) and residual disease ≥ 3 cm at the end of treatment. The MR protocol includes ECG-gated sequences Dw and Pw. The residual mass is analysed by 2 MR-experienced Radiologists evaluating 3 parameters: morphological-contrastographic features, diffusion and perfusion. The residual disease is defined as: probably active (all parameters are positive), possibly active (only 2 parameters are positive), possibly inactive (2 parameters are negative), probably inactive (all parameters are negative).We calculate the concordance rate between the analysis of 2 Radiologist. Comparisons between active disease (probably/possibly) group and inactive disease (probably/possibly) group are made.The diagnostic accuracy is calculated considering 6-month follow-up. *Results:* We enrolled 24 pts (7 HL,17 LNH).Residual desease were: 19 mediastinal, 4 abdominal, 1 lateral cervical masses. MRI and PET/CT are concordant at 19/24 sites (79,2%); the concordance rate is moderate (k=0,538). We revised 3/5 discrepant sites, 2 didn't yet undergo 6-month follow up. From the remainder 3 intrinsic discordances, 2 sites were positive on the PET/CT and probably inactive on MR and the 3 was positive on the PET/CT and possibly inactive on MR with only diffusion related parameter positive. Considering 6-month follow up, the first 2 of these sites were classified as false-positive PET error (pts were in complete remission) whereas the third was classified as false-negative MR errors (patient was in partial remission). The concordance rate is excellent (k=1). Considering the 6-month follow up as the gold standard, the diagnostic accuracy of MR is of 67% and for PET/CT is of 60%. *Conclusions:* These preliminary results show that MR and PET/CT have a moderate concordance and MR has a diagnostic accuracy slightly higher as compared with PET/TC in the evaluation of residual masses. MR seems to be an appropriate method for the post-treatment assessment of pts affected by HL and NHL.

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MULTICENTER PROSPECTIVE OBSERVATIONAL STUDY TO ASSESS EFFICACY AND SAFETY OF IBRUTINIB THERAPY IN RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA IN REAL LIFE (R.E.P. - APULIAN HEMATOLOGY NETWORK)

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Ibrutinib is an oral covalent inhibitor of Bruton tyrosine kinase that showed significant activity in relapsed/refractory mantle cell lymphoma (MCL) in clinical trials, but in real-life routine, the efficacy and safety may not always mirror those seen in clinical trials. The aim of our study is to investigate the clinical use of ibrutinib as a single-agent in pts with relapsed or refractory MCL in a real-life context. We studied a group of 35 pts treated (or still in treatment) with ibrutinib to assess effectiveness in terms of overall response rate, complete response rate, progression free survival and adverse events (AEs). At the start of ibrutinib therapy, the median age was 80 years (range, 45-82), 100% of pts had high risk MCL according to the MIPI score, 82.8% of pts had disease stage III or higher. 30 pts were treated for relapsed MCL, 5 for refractory disease. They had received a median of 2 (range, 1-5) prior regimens including different chemo-immunotherapy schemes, ASCT and newer agents such us bortezomib, lenalidomide, temsirolimus. We observed 7 complete responses, 1 after only 2 months of therapy, the others within 9 months of therapy. After 15 months, we observed 5 relapses (one of them presented central nervous system involvement) and 9 progression. 80% of pts treated for refractory disease presented progression within 6 months. The most common AEs were fatigue (11% of pts) and weight

increase (11% of pts), followed by diarrhea and bleeding (grade ≤ 2) (5.7% of pts). The most common hematologic event observed was neutropenia (8.6% of pts, grade ≤2). With an estimated median follow-up of 6 months (range, 4 to 30), 21 pts are still receiving treatment, 14 have discontinued therapy for relapse or progression of disease. Follow-up is still ongoing. Our preliminary data demonstrate that ibrutinib shows a high response rate and produces rapid responses regardless of the number and quality of prior regimens. However, the quality and time of response does not seem to be predictive of a better PFS or longer duration of response. Furthermore, resistance to ibrutinib in pts with MCL is associated with fulminant, severe progression. Ibrutinib is weel tolerated also in real-life experience. The weight increase in 11% of pts suggests that ibrutinib may have an anabolic effect, including alterations in blood pressure and lipid profile. Larger cohorts of pts and longer follow-up are warranted to confirm these preliminary data.

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THE USE OF DEAUVILLE FIVE POINTS SCORE REDUCES THE RISK OF FALSE POSITIVE FDG-PET IN THE POST THERAPY EVALUATION OF PATIENTS WITH PRIMARY BONE LYMPHOMA (PBL)

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Purpose: Primary bone lymphoma (PBL) is a rare disease. Little is reported about response evaluation procedures in these patients. Our aim was to evaluate response to therapy according to FDG-PET results, and in particular to test the Deauville 5-point scale as compared to the visual evaluation of FDG-PET scans in PBL. *Methods:* We diagnosed in a single center 31 consecutive patients with PBL, 24 were evaluated with end-of-treatment FDG-PET. Patient ages ranged from 19 to 82 years. Six patients were treated with chemotherapy, 24 with chemotherapy and radiation therapy and 1 patient with radiotherapy alone. Six patients were affected by a pathological fracture. Four patients died within the range of three to 36 months after diagnosis. Average follow-up of the remaining patients was 70 (24–173) months. *Results:* Overall survival was 87% at five years. The only positive prognostic factor was complete remission after chemotherapy. According to visual criteria, end-of-treatment FDG-PET was evaluated in 24 patients and it was positive in 11 (46%) and negative in 13. We organize a retrospective central blinded revision of end-of-therapy FDG-PET scans using the 5-point Deauville Score (DS). We reviewed 17 out 24 patients and we obtained the following *results:* at the end of therapy 13 patients with DS 2, 3 patients with DS 3, 1 patient with DS 4 and none with DS 5. Considering that all 24 patients achieved complete remission after treatment, visual interpretation produced 11/24 false positives results, and DS interpretation produced 1/17 false positive results, thus significantly reducing the number of false positives. *Conclusions:* In PBL the final evaluation at the end of therapy with FDG-PET should be evaluated using Deauville 5-point scale in order to reduce significantly the risk of false positive scans. *Aknowledgment:* This study was supported by the Fondazione Biagioni Borgogni O.N.L.U.S.

Chronic Lymphocytic Leukemia 1

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A PYRAZOLO[3,4-D]PYRIMIDINE COMPOUND INHIBITS FYN TYROSINE KINASE PHOSPHORYLATION AND INDUCES APOPTOSIS AND CELL CYCLE ARREST IN DIFFERENT HEMATOLOGICAL MALIGNANCIES, PARTICULARLY IN NK NEOPLASTIC DISORDERS

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Target-therapies are based upon drugs acting on tumors by interfering with specific molecular pathways involved in growth and spread of cancer. Many of such therapies have been recently approved as standard treatments in hematological malignancies (HMs), others are currently investigated into preclinical or clinical studies. The lack of effective treatments in some HMs and the development of drug-resistance in other ones emphasize the need for searching new molecular targets and therapeutic agents. Our studies led us to identify 4c pyrazolo[3,4-d]pyrimidine compound capable of inhibiting Fyn tyrosine kinase activation and inducing apoptosis in several cancer cell lines. This tyrosine kinase has a key role in different biological processes, such as cell growth and differentiation, and also in other pathological mechanisms of HMs. The effect of 4c compound was evaluated on both lymphoid and myeloid neoplasms. We demonstrated its ability to reduce cell viability, induce apoptosis and cell cycle arrest in lymphoid cell lines such as Jurkat, SKM-M1, Derl-2, Derl-7, and myeloid cell lines, such as Jurl-MK1. Moreover, we reported a high expression of Fyn in these cell lines compared to healthy subjects. In depth, we investigated Fyn expression and the effect of its inhibitor in natural killer (NK) malignant cells isolated from leukemic patients or in NK cell lines. NK cell neoplastic disorders are characterized by clonal proliferation of cytotoxic NK cells. Since there is no standard treatment to date, new therapeutic options are needed, especially for NK aggressive tumors. Firstly, we showed Fyn marked over-expression in NK leukemic cells compared to peripheral blood mononuclear cells from healthy donors. Subsequently, we demonstrated that 4c treatment reduced cell viability and induced caspase 3-mediate apoptosis, as well as cell cycle arrest in NK cells. Moreover, by inhibiting Fyn phosphorylation, 4c compound decreased Akt and P70 S6 kinase activation and changed the expression of genes involved in cell death and survival in NK cell line. Our study demonstrates that Fyn could be involved in the pathogenesis of NK leukemias and, therefore, it could represent a new potential target for these HMs. Moreover, we proved that Fyn inhibitor pyrazolo[3,4-d]pyrimidine compound might be a started point to develop new therapeutic agents.

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LDH AS PREDICTIVE PARAMETER IN TREATMENT-NAÏVE PATIENTS WITH TRISOMY 12 CHRONIC LYMPHOCYTIC LEUKEMIA

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Patients affected by chronic lymphocytic leukemia (CLL) that have trisomy 12 (+12) on FISH analysis have unique clinical and biological

features. We tried to identify parameters that predict disease progression, time to treatment and survival in treatment-naive patients with +12 CLL. Our study included 487 treatment-naive patients with +12 CLL from 16 academic centres, diagnosed between 2000 and 2016. A cohort of 250 patients with +12 CLL followed at a single US institution was used as external validation. Parameters associated with shorter progression free survival (PFS), treatment free survival (TFS), overall survival (OS) and CLL-specific survival (considering only the deaths due to haematological disease) on univariate analysis were IGHV, LDH, -2-microglobulin and Rai stage; age, ZAP70 and CD38 associated with OS only. On multivariate analysis, high LDH and unmutated IGHV remained significantly with shorter PFS, TFS, OS and CLL-specific survival, higher Rai stage with shorter PFS and elevated -2-microglobulin with shorter OS. Considering interestingly the association of a simple laboratory parameter such as LDH to the outcomes, confirmed on multivariate analyses for PFS (hazard ratio [HR] 1.55, 95% confidence interval [CI] 1.2 to 2.1; p=0.004), TFS (HR 1.62, 95% CI 1.2-2.2; p=0.002), OS (HR 1.69, 95% CI 1.1-2.7; p=0.034) and CLL-specific survival (HR 3.86, 95% CI 2.0-7.5; p<0.001), we divided our +12 CLL cohort according to LDH levels at diagnosis: 103 patients showed LDH levels above the normal limit and 184 within normal range. Patients with high LDH levels showed shorter PFS (30 months *vs* 65 months, p<0.001; Figure 1A), TFS (33 months *vs* 69 months, p<0.001; Figure 1B), OS (131 months *vs* 181 months, p<0.001; Figure 1C) and CLL-specific survival with a rate of attributable mortality of 29% *vs* 11% (p<0.001). In the validation cohort, 104 patients had high LDH levels and 145 patients had normal LDH levels; factors significantly associated with PFS and TFS on univariate analysis were LDH, -2-microglobulin, Rai stage and ZAP70; LDH, -2-microglobulin and age associated with OS. On multivariate analysis high LDH was the sole parameter significantly associated with all shorter outcomes, along with elevated -2-microglobulin, associated with shorter OS. Our study on 487 patients with +12 CLL and the analysis on 250 patients of the validation cohort showed that patients with +12 and elevated LDH have shorter PFS, TFS, OS and CLL-specific survival.

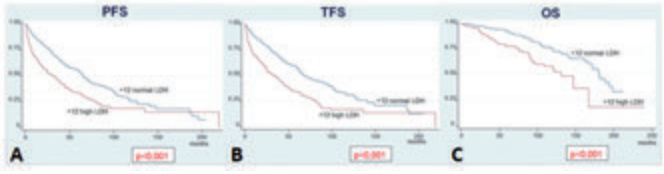

Figure 1.

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TARGETING HIF-1 AND ITS REGULATORY PATHWAYS AS A STRATEGY TO HAMPER TUMOR-MICROENVIRONMENT INTERACTIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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The CXCL12/CXCR4 axis has a fundamental role in the microenvironment-mediated protection of chronic lymphocytic leukemia (CLL) cells from spontaneous and drug-induced apoptosis. The binding of CXCL12 to CXCR4 activates RhoA- and Ras-dependent signalling. We have previously shown that co-culture with stromal cells (SC) induces in CLL cells the activation of RhoA/RhoA kinase and Ras/ERK1-2 signalling, the upregulation of Akt, and an increased activity of the transcription factor HIF-1. The purpose of this study was to identify new possible pharmacological targets involved in the CXCL12/CXCR4 axis in order to impair the protection exerted by SC towards spontaneous and fludarabine-induced apoptosis in CLL cells. Patient-derived CLL cells were cultured alone or with murine M2-10B4 SC. Patient-derived bone marrow SC were also generated. In selected experiments, recombinant CXCL12, CXCR4 inhibitor AMD3100, fludarabine, simvastatin, ERK1-2 kinase inhibitor PD98059, HIF-1 inhibitor BAY87-2243, or PI3K-delta inhibitor idelalisib were added. We analysed the activity of Ras, RhoA, RhoA kinase, Akt and HIF-1, the expression of ERK1-2, the expression and phosphorylation of HIF-1, the CXCL12 production, and CLL cell viability. The exposure of CLL cells to recombinant CXCL12 led to the activation of RhoA- and Ras-dependent signalling, and to the downstream upregulation of HIF-1. The CXCR4 antagonist AMD3100 completely abrogated the positive regulation exerted by both CXCL12 and SC, thus unveiling the key role of the CXCL12/CXCR4 axis in the SC-induced modulation of these signalling pathways. The inhibition of Ras and RhoA activity by simvastatin, the inhibition of ERK1-2 and HIF-1 by PD98059 and BAY87-2243, and the targeting of the PI3K/Akt pathway with idelalisib effectively blocked the SC-induced expression and activity of HIF-1, significantly impairing the SC-mediated protection of CLL cells, both in absence or presence of fludarabine. At the SC level, simvastatin and BAY87-2243 effectively inhibited HIF-1 expression both in M2-10B4 and in patient-derived SC. Moreover, simvastatin significantly reduced the secretion of CXCL12, which is a known transcriptional target of HIF-1. Our data demonstrate that the targeting of HIF-1 and its regulatory pathways - both at the tumor cell and at the SC level - is an appealing strategy to overcome the microenvironment-mediated protection toward spontaneous and fludarabine-induced apoptosis in CLL cells.

P166

DEVELOPMENT OF A SCORING SYSTEM TO PREDICT THE RISK OF ATRIAL FIBRILLATION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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The B-cell receptor inhibitor Ibrutinib significantly changed treatment and management of chronic lymphocytic leukemia (CLL). Although, several data in the literature suggest that ibrutinib increase the risk of atrial fibrillation (AF), the incidence of AF in a general cohort of CLL patients is unknown. The aim of this study was to describe the prevalence and risk factors of AF in ibrutinib-naive CLL and to define a predictive model for the development of AF. We retrospectively analyzed data of 860 CLL patients, followed at Padua University hospital. Comorbidities, clinical and biological prognostic markers were analyzed using Mann-Whiney, Fisher exact or Chi-square, when appropriated. The rate of AF during the follow-up and overall survival (OS) were evaluated with Kaplan-Meier methods. Univariate and multivariable Cox models were run to identify independent factors associated with AF. Then, risk values were obtained based on the hazard ratios. The score for AF was calculated as the sum of each risk values. A prior history of AF was present in 21 (2.4%) patients at CLL diagnosis. While, among the 839 patients without a history of AF, 47 (5.6%) developed it after a median follow-up of 9.4yy, resulting in an estimated incidence of 0.8%cases/year.

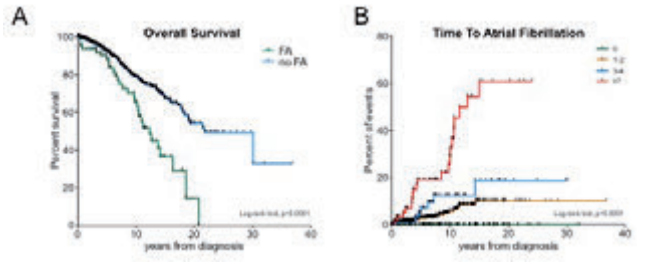

Figure 1.

Moreover, the median OS for patients with AF was significantly shorter than those without AF (12 *vs* 22yy, p<0.0001, Fig 1A). Age>65yy (p=0.001), male gender (p=0.003), valvular hearth diseases (p=0.001), cardiopathy (p<0.001), hyperthyroidism (p=0.001), chronic lung diseases (p=0.001), diabetes mellitus (p=0.023), severe infections (p=0.019)

were associated with the risk of AF on multivariate analysis. As expected, no clinical and biological prognostic markers (*i.e.* Rai stage, IGHV mutation, TP53 abnormalities) for CLL were linked with an increase risk of AF. A predictive model for developing AF designed from these factors stratifies patients into 4 different groups. The estimated incidences of AF after 15yy of follow-up were 0%, 10%, 19% and 61% for patients with score 0, 1-2, 3-4, and ≥5 respectively (p<0.001, Fig 1B). In this study, some variables linked to an increased risk of developing AF were identified and recapitulated into a scoring system model. Interestingly, 3 out of 23 subjects who received ibrutinib developed AF and all had a score ≥5. Taking these preliminary data into account, patients with a score ≥5 should be carefully monitored during ibrutinib treatment given the very high-risk to develop AF.

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B-CELL RECEPTOR INHIBITORS ARE ACTIVE AND EFFECTIVE DRUGS IN RELPASED/REFRACTORY RICHTER SYNDROME

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Richter syndrome (RS) is a rare complication of chronic lymphocytic leukemia (CLL), characterized by quickly growing lymphadenopathy or extranodal masses, high lactate dehydrogenase and hypercalcemia. Most RS represent transformation to activated B-cell type diffuse large B-cell lymphoma and a small proportion of Hodgkin lymphoma. Less than 50% patients respond to first-line treatment and only a few of them are candidate to stem cell transplantation. The advent of B-cell receptor inhibitors (BCRi), Ibrutinib and Idelalisib, significantly changed management and the clinical course of CLL. The aim of this study was to assess the activity of the above-mentioned BCRi in relapse/refractory RS. Only histologically confirmed RS patients were included in the study. Criteria for exclusion were i) Hodgkin lymphomas ii) need for treatment with strong inhibitors of CYP3A4, iii) contraindication to receive BCRi. The primary endpoint was progression free survival (PFS). Among the 22 cases of RS followed at Padua University hospital, 7 were treated with BCRi at treatment failure. Four out of 7 subjects were managed with Ibrutinib at 420mg/die and the remaining 3 with Idelalisib 150mg bid plus Rituximab, based on patients comorbidities. The median age at starting treatment were 70 years (range 56-81), 4 patients were males and 6 had more than two comorbidities. According to Tsiberidou's score patients were classified as 1 low-intermediate, 4 high-intermediate and 2 high risk. While, according to Rossi's models 4 subjects were at intermediate-risk and 3 were at high-risk. The median numbers of previous treatments was 1.9 (range 1-4). After a median follow-up of 67 days (range 11-313 days) from starting BCRi 4 subjects achieved a partial response, 2 stable diseases and 1 progressed. The median PFS was not reached and the estimated 3-months PFS was 85%. Two patients died during the follow-up, one due to disease progression and one of sudden death in partial response. One patient developed paroxysmal atrial fibrillation during Ibrutinib; therapy was not stopped and was managed with new oral anticoagulants. None of 3 patients under treatment with Idelalisib developed colitis or pneumonitis. We herein report that new BCRi are active and effective drugs in RS. Although a longer follow-up is needed, these preliminary data give new hope for patients with relapsed Richter syndrome and offer the rationale for designing clinical trials incorporating BCRi in first line therapy.

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PROGNOSTIC AND PREDICTIVE ROLE OF IGHV MUTATIONS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA: ANALYSIS ON 527 SUBJECTS

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Prognostic and predictive markers are commonly used to identify patients with increased risk of progression and death or early relapse after treatment, respectively. Most important prognostic markers in chronic lymphocytic leukemia (CLL) are 17p deletion, TP53 mutations and IGHV mutations. The aim of this study was to investigate the prognostic and predictive role of IGHV mutation in treatment-naive (TN) and relapse/refractory (R/R) patients with (CLL). Among the 816 CLL patients followed at the Hematology Unit of Padua from 1989, 527 had productive rearrangement of the B-cell receptor. IGHV mutational status was tested at CLL diagnosis. Homology >98% of IGHV gene from the germline sequence identifies unmutated cases (U-IGHV), otherwise patients were considered mutated (M-IGHV). Survival curves were compared with log-rank test and plotted using Kaplan-Meier method. The prognostic activity was assessed in the whole cohort of 527 patients. The median progression free survivals (PFS) were 2.9 and 15.7 years for U and M-IGHV patients, respectively (p<0.0001, Figure 1A). Moreover, U-IGHV subjects had a shorter overall survival (OS) compared to M-IGHV (11.5 *vs* 30.1 years, p<0.0001). To evaluate the predictive strength of IGHV mutational status we analyzed 256 patients who required treatment during the follow-up. Among all first-line treated patients, those with U-IGHV had almost 2-fold increased risk of relapse and death than M-IGHV subjects (hazard ratio 1.9 and 2.4, respectively, p<0.0001). Focusing on 64 TN patients who received fludarabine-cyclophosphamide-rituximab (FCR) chemoimmunotherapy, the median PFS was 3.9 years for U-IGHV but not reached for M-IGHV patients (p=0.0328, Figure 1B). Conversely, the predictive strength of IGHV mutations was lost when considering all R/R patients or those treated with FCR (1.8 *vs* 0.9 years, p=0.8479, Figure 1C) and bendamustine-rituximab (BR, 1.8 *vs* 3.3 years, p=0.1568, Figure 1D). According to data from the literature IGHV mutations were able to identify patients with early progression and at increased risk of death. In addition, we shed light on the predictive strength of IGHV mutations, demonstrating their usefulness for first line treatment but not for further lines.

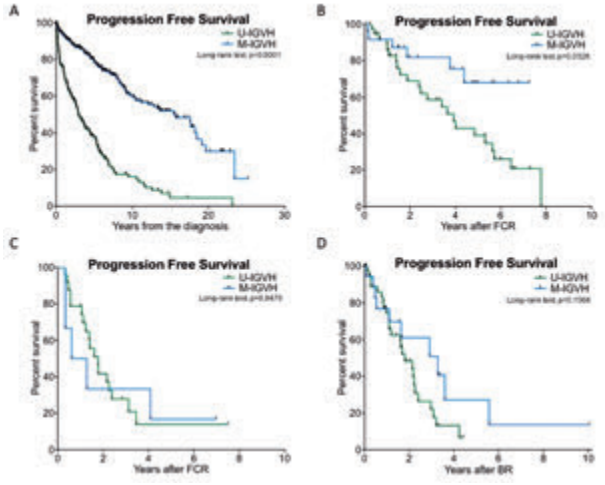

Figure 1.

P169

TREATMENT WITH IBRUTINIB AND IDELALISIB IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA: REPORT FROM THE HEMATOLOGICAL NETWORK OF THE VENETO REGION (REV)

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The B-cell receptor and cytokines signaling have pivotal role in chronic lymphocytic leukemia (CLL) promoting cell survival, proliferation and microenvironment interaction. BTK and PI3K are critical partners at the interplay between these pathways. In fact, inhibitors of the above two kinases, Ibrutinib and Idelalisib, were proven to be active and effective in phase 3 trials. However, there are only few data from daily clinical practice. The aim of this study was to retrospectively collect data from CLL patients treated with Ibrutinib and Idelalisib by the Veneto Hematological Network. The primary endpoint was progression free survival (PFS). Secondary endpoints included overall survival (OS), PFS and OS among patients with TP53 abnormalities and IGHV mutation. Ibrutinib was administrated at 420mg/day and Idelalisib at 150mg BID in combination with Rituximab according to Furman's protocol, depending on physician choice. Data were recorded from 81 CLL patients (67 received Ibrutinib and 14 Idelalisib), 6 were treatment naive (5 Ibrutinib and 1 Idelalisib), 57 were males and 24 females. The median age was 69 years (range 47-87years), the median number of previous treatment was 3 (2.8 Ibrutinib and 3.7 Idelalisib, p=0.2403) and the median duration of response to last treatment was 14 months (13 Ibrutinib and 15 Idelalisib, p=0.9882). Two patients developed Richter's syndromes, 4 atrial fibrillations, 4 G3-4 bleedings, 12 G3-4 infections (7 Ibrutinib and 5 Idelalisib) but no colitis and pneumonitis. After a median follow-up of 10 months 13 patients progressed (7 Ibrutinib and 6 Idelalisib) and 13 died (7 Ibrutinib and 6 Idelalisib). Median PFS were 33 and 21 months for Ibrutinib and Idelalisib, respectively (p=0.0003). The estimated 12-month OS were 89% and 61% for Ibrutinib and Idelalisib, respectively (p=0.0022). Treatment with Ibrutinib showed a 33% reduction in mortality as compared with Idelalisib. Considering each drug separately, both PFS and OS were independent from TP53 abnormalities, IGHV mutational status, number of previous lines of treatment and need of dose reduction. In this study we performed the first retrospective direct comparison between Ibrutinib and Idelalisib, and demonstrated that former is more commonly used in the daily clinical practice. In addition, our analysis seems to suggest a better disease control with Ibrutinib. However, this consideration should be taken with caution since Idelalisib-treated patients group was smaller.

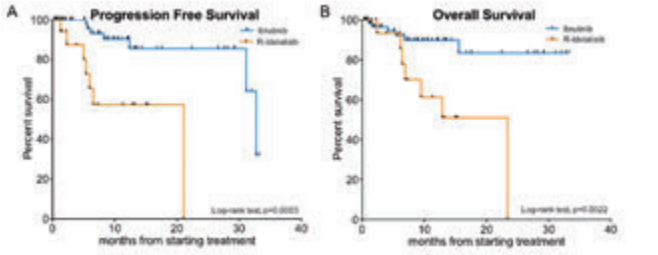

Figure 1.

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CLADRIBINE VERSUS PENTOSTATINE IN CLASSIC HAIRY CELL LEUKEMIA TREATMENT: A SINGLE-CENTER EXPERIENCE ON A LONG-TERM FOLLOW UP

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Hairy Cell Leukemia (HCL) is a chronic mature B-cell neoplasm characterized by a peculiar morphology of tumor B cells (abnormal hair-like projections of cytoplasm) and by a specific immunophenotype (CD11c+,CD25+,CD103+). The 2008 WHO classification distinguishes a classic HCL (HCLc) and a rare form referred to as HCL variant. The landscape of HCL changed in 2011 with the discovery of the BRAF V600E mutation as a molecular hallmark of HCLc. The prognosis of HCL has improved with the introduction of purine analogs (Pentostatin and Cladribine [2CDA]) and the possibility to detect BRAF mutation which can be a new therapeutic target. The aim of this study was to compare the long-term efficacy of the Pentostatin and 2CDA in HCLc patients from a single institution. We perform a retrospective study on 51 patients (10 females, 41 males), admitted at the Hematology Division of Padua. The diagnosis was based on 2008 WHO criteria and all patients were positive for BRAF mutation. Median age at the diagnosis was 52 years (34-74). 17 patients (33%) were treated with 2CDA with different schedules of administration (IV continuous infusion for 7 days; IV infusion for 2 hours on a 5-day regimen, or alternatively SC on a once-per-day or once-per-week regimen); according to the literature, these schedules provide comparable efficacy. Thirty-four patients (67%) were treated with Pentostatine IV weekly for the first 6 weeks, then every 2 weeks for the last two doses. During a median follow up of 113 months (range 3-228) 11 patients relapsed and 3 died (1 for systemic aspergillosis, 2 for metastatic cancers). The estimated 5-year progression free survival (PFS) and OS for the whole population were 77% and 92%, respectively. According to data from the literature patients who achieved CR have better median PFS as compared to those who did not achieve CR (median PFS not reached *vs* 53 months; p<0.0001). We also found a higher CR rate with 2CDA than Pentostatine (Fisher's exact test p=0,0348), but no difference in OS (94% *vs* 87%). Our data seem to suggest that patients treated with 2CDA have a longer PFS as compared to those treated with Pentostatine but a statistically significant difference was not reached, likely due to the low number of subject receiving 2CDA (5-yrs PFS 93 *vs* 71 months; 10-yrs 75 *vs* 53 months; p=0.1991). In conclusion, both 2CDA and Pentostatin appear a reasonable approach in HCL. In our experience, 2CDA treatment allows higher CR rate and perhaps longer PFS.

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IDIOPATHIC PULMONARY HYPERTENSION AND LARGE GRANULAR LYMPHOCYTE LEUKEMIA: TURN ON THE LIGHT ON THE ASSOCIATION BETWEEN THESE TWO RARE DISEASES

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Large Granular Lymphocyte Leukemia (LGLL) and Idiopathic Pulmonary Hypertension (IPAH) are rare disorders which have been reported to be sometimes associated. In these cases a direct role of LGL has been suggested, mediated through a direct cytotoxic effect on pulmonary endothelial cells. We herein report three cases of LGLL in patients with IPAH. LGL proliferation was characterized in two cases by cells expressing the CD3+CD8+CD16+CD57+CD56- immunophenotype, which is indicative for T-LGLL, whereas in the third one the diagnosis of NK-CLPD was established according to the CD3-CD16+CD56+ LGL phenotype. Both CD3+ patients showed STAT3 mutations (one Y640F and one D566N) with STAT5b wild type whereas NK-CLPD patient showed STAT3 and STA5b wild type. The first T-LGLL patient received Cyclosporine A 150 mg/die for severe neutropenia and this therapy, in combination with Sidenafil 60 mg/die, well controlled the pulmonary hypertension (mean pulmonary arterial pressure, mPAP, from 55 to 37 mmHg, functional class, FC, from NYHA III to NYHA II). The second T-LGLL patient started treatment with Methotrexate 15 mg/week for symptomatic anemia obtaining a relevant respiratory and hematological improvement. During the follow up, a reappearance of symptoms associated to an increase of pulmonary pressure was documented and the therapy was shifted to Cyclophosphamide 50 mg/die and Ambrisentan 5 mg/die which led to a rapid recovery of respiratory function (mPAP from 47 to 30 mmHg, FC from NYHA II to NYHA I). The NK-CLPD patient received a combination of Bosentan 250 mg/die, Sindenafil 90 mg/die and Nifedipine 30 mg/die for her IPAH achieving a good control of the respiratory symptoms (mPAP from 65 to 50 mmHg, FC from NYHA III to NYHA II). According to the good hematological condition, immunosuppression was not started in this patient up to now. At the last evaluation (January 2017) all three patients were in good health conditions, after an average fol-

low up from IPAH diagnosis of 16 years. Provided that 43% of patients with IPAH die after seven years from diagnosis despite specific treatment, these findings suggest that in case of association with LGL proliferation, clinical course of IPAH is more indolent and might respond to the immunosuppressive therapy set for LGLL. According to this hypothesis, the evaluation of lymphoid subpopulations in IPAH patients is suggested in order to identify a putative misdiagnosed LGL lymphoproliferation.

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CLL-IPI AS A TOOL TO ASSESS OVERALL SURVIVAL OFCLL PATIENTS? A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: A weighted grading approach based on five independent prognostic factors (i.e, TP53 status, IGHV mutational status, ß2-microglobulin, clinical stage and age) has been used by an international Working Group to generate the chronic lymphocytic leukemia international prognostic index (CLL-IPI). We conducted a systematic review which includes all published studies which used CLL-IPI to prognosticate overall survival (OS) in CLL. *Material & Methods:* A comprehensiveMEDLINE search using "CLL-IPI"as Medical Subject Headings (MESH) allowed to identifyat the cut-off time of February the 28, 2017 "seven hits" with only "four" citations considered pertinent. The search extended to the conference proceedings of annual meetings of ASH, EHA and ASCO of last two yearsrecognized "three" additional citations.

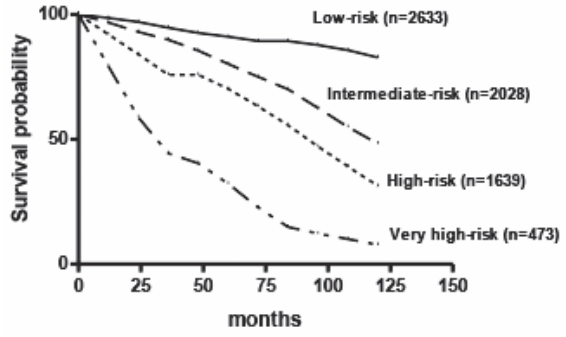

Figure 1.

Results: Overall 6720 patients from sevenevaluable studies were suitable for the present analysis aimed at assessingthe impact of CLL-IPI on OS.The majority of patients (4953 or 73.7%) came from studies of external validation of CLL-IPI while 17% (1192) and 8.5% (576)had been used to generate (training set analysis) and to internally validate the model. Patient distribution into the four risk categories of CLL-IPI was heterogeneous thus reflecting the CLL phase (*i.e.*, at diagnosis, at time of first treatment and at relapse) of patients within different studies. Accordingly, patients diagnosed as having low-, intermediate-, high- and very high-risk CLL-IPI ranged respectively between 9% and 58%, 25% and 39%, 14% and 52% and 2% to 9%. Next we evaluated the 5-year OS of patients stratified into each of the four CLL-IPI risk groups using either "Q" or "I2" test to assessthe heterogeneity across different studies. The 5-year survival probability was 91% for low-risk group (95% CI, 90-91%; Q=55.2; P< 0.00; I2, 87%), 80% for intermediate-risk group (95% CI, 79-82%; Q=49.36; P<0.00;I2, 86%), 60% for high-risk group(95% CI, 57-62%; Q=42.78; P<0.00; I2, 84%) and 32% for very high-risk group (95% CI, 27-38%; Q=18.1; P=0.01; I2, 67%). *Conclusions:* In this comprehensive review and meta-analysis of studies thus far published on CLL-IPI we confirmed the value of this novel model to predict OS whatever the CLL phase (fig 1). The prognostic impact of CLL-IPI needs an extensive validation in patient cohorts receiving therapy with B-cell receptor or bcl-2 inhibitors. Nonetheless, in a study of relapsed/refractory CLL included in this analysis the PI3K-inhibitor idelalisib was not able to overcome the impact of CLL-IPI risk categories on OS.

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PLATELET DYSFUNCTION DURING IBRUTINIB IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA. CLINICAL AND LABORATORY CHARACTERIZATION IN A MONOCENTRIC EXPERIENCE

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Ibrutinib (IBR) is a Btk inhibitor for the treatment of chronic lymphocytic leukemia (CLL) with del17p/TP53 mutation or relapsed/refractory (R/R) CLL. IBR is associated with bleedings usually mild (I-II grade), rarely severe (III- IV grade), probably secondary to a defect of platelet function caused by an inhibition of Btk-mediated signaling by platelet glycoproteins (GP) GPVI and GPI. Bleedings and platelet dysfunction may be relevant in CLL patients (pts) who are usually elderly and with comorbidities. We enrolled 20 pts with CLL treated with IBR; 18 R/R pts received only IBR and 2 treatment-naïve pts received IBR with anti-CD20 MoAb. Median age was 68 years (57-84); 13 pts had unmutated IgVH and 2 had 17p deletion. Five pts discontinued IBR for: Richter's transformation(2), progressive CLL(1), allo-HSCT(1), heart disease(1). All pts at the baselines and during the treatment with IBR were studied with light transmission aggregometry (LTA) using platelet-rich plasma and the following agonists: ADP 2-4uM, PAR1-AP 25uM, Collagen 10-3.3-2 ug/mL, arachidonic acid 1 mM, ristocetin 0.6-1.2 mg/mL; measurements of von Willebrand factor antigen (vWF:Ag) and ristocetin cofactor activities (RiCo) by chemiluminescent immunoassay were performed. No patient received concomitant antiplatelet or anticoagulation therapy. In 15 pts we observed just mild events (bruising, petechiae, conjunctival and retinal hemorrhage, rectal bleeding); no patient interrupted or reducted IBR. All pts displayed severe impairment of collagen induced aggregation upon IBR. In all pts during IBR was measured a reduction of maximal aggregation (36+/-32% *vs* 71+/-21%) and prolongation of the lag phase (261+/-54 sec *vs* 72+/-27) by 2 ug/mL collagen. In 10 pts was found a relevant improvement of the aggregation by 2 uM ADP (71+/-32% *vs* 49+/-31%) and 4 uM ADP (84+/-11% *vs* 64+/-25%). The aggregation by 25 uM PAR1-AP, 1.2 mg/ml ristocetin and 1 mM arachidonic acid was unchanged before and under IBR. Finally, in 9 pts the vWF:Ag and RiCo were high at the onset of the disease (163+/-60% and 182+/-82%) and reduced up to normal values under IBR(118+/-71% and 145+/-65%). A severe impairment of collagen-induced aggregation was caused by IBR, with an amelioration of ADP-induced aggregation, that could explain the mild clinical phenotype in treated pts. The assessment of platelet function in IBR treated CLL pts could help to predict and monitor the bleeding risk, and to guide pts through invasive procedures.

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IBRUTINIB USE FOR RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA IN A PATIENT WHO UNDERWENT ALLOGENEIC BONE MARROW TRANSPLANTATION FOR OTHER REASONS: A CASE REPORT

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Ibrutinib (Ibr), an inhibitor of Bruton's tyrosine kinase, has shown great efficacy in patient with high risk or relapsed/refractory chronic lymphocytic leukemia (CLL) and there are promising data about its use as a salvage therapy for CLL that relapsed after allogeneic hematopoietic stem cell transplantation (alloHSCT). Treatment with Ibr also resulted in relevant and durable responses in refractory chronic graft-*versus*-host diseases (GvHD) and possible enhancement of the graft-*versus*-leukemia (GvL) effect. We present a case report showing that Ibr can be safely used to treat CLL in patients who underwent alloHSCT for other malignancies. CP is nowadays a 76 years old lady who was diagnosed with a high-risk IgA multiple myeloma (MM) that achieved a partial response and underwent a non-myeloablative HLA-matched alloHSCT in 2000 with her brother as the donor. After transplant, she achieved full donor chimerism and a complete response from MM. Subsequently, she developed chronic GvHD involving liver, eyes, mouth and skin that was controlled with immunosuppressive therapy. In 2004, the donor developed a CLL that showed a 46, XY karyotype with a microdeletion at the 13q14.3 locus. In 2010, CP developed leukocytosis that was investigated with immunophenotype and cytogenetic analyses. The results were consistent with a typical CLL phenotype (CD19+, CD5+, CD20dim+, CD22dim+, CD23+, fmc7-, CD38-). Cytogenetic analysis showed a male karyotype and a microdeletion at the 13q14.3 locus, confirming a donor cell leukemia. Approximately two years after diagnosis, our patient was classified as stage I Rai and B Binet and underwent intermittent treatment with chlorambucil. In February 2016, she relapsed and developed significant B symptoms, thrombocytopenia and lymphocyte doubling time lower than 3 months, meeting the criteria to begin a new treatment. Treatment with Ibr was then started. After more than one year of treatment, she is responding to therapy (currently stage 0 Rai) and continues daily Ibr therapy. Ibr is well tolerated with minor side effects treated with symptomatic therapy. Her GvHD is stable and nonprogressive on Ibr therapy without any need for additional specific therapy. In conclusion, our findings confirm that Ibr is a safe and effective drug for relapsed CLL and suggest the feasibility of its use in patients who underwent alloHSCT for other reasons because of its efficacy, minor side effects profile and its putative benefit in both GvHD and GvL effect.

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MONOCENTRIC REAL LIFE COHORT OF UNSELECTED CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS (CLL): ANALYSIS OF BIOLOGICAL AND CLINICAL FEATURES

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Chronic lymphocytic leukemia (CLL) is a heterogenous disease with a wide variability in presentation and course, while some patients never need treatment whilst many others require multiple lines of therapy during their lifetime and often die from disease. We analyzed a monocentric real life cohort of 156 unselected CLL patients with a long term follow up (median: 7 yrs; range: 1-28) for biological and clinical features and their impact on overall survival (OS) and time to progression (TTP). All patients, 63 untreated (40%) and 93 treated patients (60%), have been observed in our institution from 01/1988 to 02/2017; 90 were male and 66 female; median age at diagnosis was 63 (37-88). At diagnosis all patients have been analyzed for Binet stage (A 106 (68%), B 31 (20%), C 19 (12%) and surface immunophenotype. Moreover, IGHV mutation (93 patients, 59%), CD38 expression, FISH, NOTCH1 and line of therapies (first, second, third/multiple lines) have been investigated. IGHV amplification was performed in 93 treated patients, with 33% unmutated *versus* 67% mutated. CD38 expression was analyzed in 145 patients (93%), with 24 CD38+ patients (15%). FISH analysis was performed before first line therapy in 83 patients (53%) and repeated in 40 cases (relapsed/refractory patients). NOTCH1 c.7544_7545delCT alleles were investigated in all 156 patients, with NOTCH1 mutation in 17 patients (11%). In 7/17 patients (41%) Richter syndrome occurred with fatal outcome and only 1 long-surviving patient. Treated patients have been divided in 3 subgroups (fluda-based, non fluda-based, new treatments) for first line (30%, 64%, 6%, respectively), second line (25%, 66% 9%, respectively) and third/multiple therapies (20%, 63% 17%, respectively). OS was 85,5 months and 85,6 months in treated and untreated patient group, respectively ($p<0.006$). Moreover, we investigated OS in treated patients with stage A (84.5 months) and stage B and C at diagnosis (18 months) ($p<0.0001$). Median time to progression (TTP) was 38 months for all treated patients, regardless of stage at diagnosis. CLL primarily affects the elderly and, as clinical trials have traditionally excluded older, it is difficult to estrapolate their findings into clinical practice. Our real life results support the use of stratification system based on known clinical and biological factors and encourage the introduction of innovative genetic lesion analysis with the aim of selecting high risk *vs* low risk patients.

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A SIMPLE FLOW CYTOMETRIC SCORE BASED ON CD49D AND HOMING MARKER EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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The discovery of new molecular markers and cytogenetic abnormalities improved risk stratification of chronic lymphocytic leukemia (CLL) patients. Ninety-four CLL patients were evaluated to con-firm the prognostic role of CD49d and to elaborate a flow cytometric score as an additional tool for the definition of CLL outcome. The scoring system was designed combining 5 membrane markers: CD5, CD20, CD11c, CD38 and CD49d. Based on previous studies, cut offs of positivity were set at 30% or 45%, and a value of 0 or 1 was given accordingly. We identified patients with favorable phenotype (FP) when the score was ≤2, or unfavorable phenotype (UP) when ≥3. Eighty CLL sub-jects showed FP: 75% of them had early stage disease and 21% $CD49d^{high}$. The remaining 14 pa-tients presented UP with $CD49d^{high}$ and 8 of them had early stage disease. Next, we sought to cor-relate our phenotypic score with chromosome abnormalities detected by FISH technique, and the risk of clonal evolution was calculated according to Rossi *et al.* study. A group of 50 CLL patients was studied by FISH: 34 and 7 CLL with very low or low risk presented favorable or unfavorable phenotype, respectively. Three of 5 patients with intermediate and all 3 with high risk disease showed FP, suggesting a different biology behind clonal evolution in high risk CLL patients. Thirty-six patients have been treated with chemotherapy, while 58 subjects have been monitored (85% of them presented FP). Twenty-five subjects received one line therapy: 92% of them achieved com-plete remission (CR, 56%) or partial remission (PR, 36%) and 80% showed FP. Nine CLL patients have been treated with two lines of therapies: 89% presented FP and all of them achieved CR (44%) or PR (56%). One patient who received a third-line treatment had FP. Of the 6 CLL treated subjects with UP, one of them received second-line therapy achieving PR; the remaining 5 patients achieved CR (80%) or PR (20%). In conclusion, our results confirm the negative prognostic role of CD49d for CLL outcome. Moreo-ver, the combination of flow cytometric score with other predetermined staging and stratification systems could better define CLL prognosis and risk of clonal evolution. Indeed, the identification of high risk phenotype with a simple scoring method could improve the treatment of these patients, who could take advantage of the most recent molecular targeting therapies.

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SPONTANEOUS CLINICAL REGRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA: CLINICAL AND BIOLOGIC FEATURES OF 9 CASES FROM THE ERIC REGISTRY

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Spontaneous clinical regression in chronic lymphocytic leukemia (CLL) is rare (Del Giudice I, 2009). In order to conduct a retrospective collection of clinical and basic biologic data on spontaneous CLL regressions (absence of lymphadenopathy, splenomegaly or constitutional symptoms, peripheral blood (PB) lymphocytes (Ly) <4x10^9/L, in the absence of any previous treatment) and to make them accessible for future research, a registry was launched within the ERIC consortium. So far, 9 CLL patients have been reported, 7 from Italy and 2 from Sweden (Tab 1). None had undergone treatment, except for one diagnosed in 2009 who received FCR for disease progression in 2013 (Ly 107x10^9/L), obtained a PR and 18 months later developed a Richter's syndrome (RS, diffuse large B-cell lymphoma clonally unrelated to CLL) with the concomitant disappearance of the CLL clone from the PB and bone marrow, that has lasted up to 2017 (Ly 3.5x10^9/L, CLL 0.035x10^9/L). An additional case diagnosed in 2013 (stage A/I, 37.2x10^9/L) reached the highest Ly count 19 months later (91.2x10^9/L) and subsequently started a spontaneous reduction in Ly down to 39.6x10^9/L in 2015 and to 8.9x10^9/L in 2017 in stage A/0, indicative of a partial but ongoing CLL regression. Excluding the latter cases, in the other 7, all in stage A/0, the highest Ly count was 16.0x10^9/L (8.9-76.0), the lowest at the last follow-up was 2.8x10^9/L (1.8-4.4), with 0.66x10^9/L CLL cells (0.085-3.0) in the 4 evaluable cases. Median time from diagnosis to clinical regression was 4 years (range 2-17) and this has been maintained for 2 further years (range 0.5-7). One case (mutated VH3-21, tris12) seems the most dramatic: in 2008 at diagnosis, the Ly were 51.9x10^9/L, in 2009 a 76.0x10^9/L peak was recorded; in 2011, when the CLL regression started, the patient underwent several mild viral upper respiratory infections; the CLL complete regression (1.8x10^9/L) persists up to the last follow-up. In 5 cases, one event - mild viral infections, a cerebral hemorrhage, a stroke, a pelvis fracture and a RS - occurred before the spontaneous regression, but no relevant drug intake was recorded. Clinicians should be aware that spontaneous regression is a possibility, albeit infrequent, in the natural history of CLL. The collection and study of such cases within the ERIC registry may shed light on mechanisms leading to spontaneous regression and critical pathways in immunosurveillance in CLL.

Table 1. Clinical and biologic features at diagnosis of the 9 CLL patients with spontaneous regression within the ERIC registry.

Gender	6 males/3 females
Median age (range)	57 years (51-82)
Stage (Binet/Rai)	6 A/0; 2 A/I; 1 B/II
Lymphocyte count (x10^9/L)	14.1 (5.3-51.9)
IGHV (mutated/ unmutated)	8/0
	2 VH3-30 1 VH3-21 1 VH3-15 1 VH3-23 1 VH4-31 1 VH4-34 1 VH4-59
CD38 (< 30%)	6/6
ZAP70 (< 20%)	4/6
FISH	0 del17p 0 del11q 1 trisomy 12 4 del13q 3 negative

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NAIL AND HAIR CHANGES DURING IBRUTINIB THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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A range of systemic drugs has been found to induce nail changes. More recently nail and hair changes have been described also in patients treated with targeted therapies, such as inhibitors of multiple tyrosine kinase receptors, BRAF inhibitors and epidermal growth factor receptors (EGFR) inhibitors. A similar toxicity has been also reported in patients treated with ibrutinib. Nail changes could be due to the ibrutinib-induced disruption of disulfide bonds between cysteines, which is critical for nail hardness. However other target kinases, as human EGFRs, could be inhibited by ibrutinib leading to the development of an impaired stratum corneum. Thirty patients with relapsed/refractory CLL were treated with ibrutinib and followed on a regular basis at our Institution. Nail changes were observed in 15/26 (58%) patients who received ibrutinib for more than 3 months. Nail lesions occurred after a median time of 8 months (range 3-24) from the start of ibrutinib treatment. Brittle nails were the most frequent abnormality that was observed in 13/15 (87%) cases. Other less common nail changes, recorded in at least 1 patient, included onycholysis, onychauxis, onychomadesis, Beau's lines, paronychia, onychocriptosis, Terry's nails, pseudoclubbing and subungueal hematoma. In the majority of cases (86%), nail toxicities were mild and queried by patients because of their cosmetic appearance and discomfort. However, a severe and painful paronychia with infection was observed in 1 patient who required systemic antibiotics and ibrutinib discontinuation. Hair thinning were observed in 2 female patients after 7 months from the start of ibrutinib. No clinical and biologic factors appeared associated with an increased rate of nail toxicities. After a median follow-up of 25 months, nail and hair changes persisted in all cases. At present, 21 patients are still on ibrutinib while 5 discontinued treatment because of disease progression (2 cases) or other toxicities (3 cases; atrial fibrillation, 1, infection, 1, persistent cytopenia, 1). Our findings show that nail changes represent in CLL patients treated with ibrutinib a bothersome, but usually mild, side effect. Patients on long-term treatment with ibrutinib should be aware of the potential nail toxicities and advised on strategies to prevent and minimize the related discomfort, such as limiting an excessive exposure to water and or chemicals, appropriate nail cutting, use of hand creams and biotin supplementation.

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REGULATION OF HIF-1 IN TP53 DISRUPTED CLL CELLS AND ITS INHIBITION AS A STRATEGY TO OVERCOME FLUDARABINE RESISTANCE

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Treatment of high-risk chronic lymphocytic leukemia (CLL) patients remains an unmet clinical need. Disease aggressiveness can be ascribed to intrinsic features of the tumor cells (*i.e.* TP53 disruption), and to the interactions of CLL cells with stromal cells (SC) of the microenvironment. HIF-1 is a transcription factor implicated in cell adaptation to hypoxia and it is involved in the regulation of genes implicated in tumor

progression. In CLL cells HIF-1 is constitutively expressed even in normoxia and modulates the interactions with SC. The aims of this study were to understand the HIF-1 regulatory pathways in TP53 disrupted (TP53dis) CLL cells and to evaluate the ability of HIF-1 inhibition to exert a direct cytotoxic effect and to potentiate fludarabine cytotoxicity toward TP53dis CLL cells. CLL cells were considered TP53dis when TP53 mutation or 17p deletion were present. Otherwise CLL cells were considered wild type (TP53wt). CLL cells were cultured alone or with the M2-10B4 SC under normoxic or hypoxic conditions and exposed to fludarabine and/or the HIF-1 inhibitor BAY 87-2243. We evaluated the activity of ERK1-2, Ras, RhoA, RhoA kinase, Akt by Western Blot or specific immunoassay kit. HIF-1 expression was assessed by RT-PCR and Western Blot. Cell viability was analyzed by AnnexinV/propidium Iodide immunostaining. TP53dis CLL cells showed a significantly higher HIF-1A gene expression compared to TP53wt CLL cells, and also had higher amount of HIF-1 protein. Accordingly, TP53dis CLL cells overexpressed the HIF-1 target genes p21, Bcl-2 and ENO1, and had a more active glycolysis than TP53wt CLL cells. Hypoxia further increased HIF-1 expression in both TP53dis and TP53wt CLL cells. Similarly, the co-culture of CLL cells with SC further upregulated HIF-1 in both subsets via the activation of Akt, Ras- and RhoA-dependent signaling cascades. BAY87-2243 efficiently inhibited HIF-1 and induced a significant reduction in viability of CLL cells in both subsets. *In vitro* fludarabine-resistant CLL cells showed higher levels of HIF-1A gene compared to fludarabine-sensitive, and the inhibition of HIF-1 with BAY87-2243 restored fludarabine-induced cytotoxicity. Lastly, HIF-1 inhibition was also able to reverse the SC-mediated resistance to fludarabine cytotoxicity in both TP53dis and TP53wt CLL cells. Based on our results, HIF-1 represents an interesting therapeutic target in CLL, in particular for the high-risk disease subset.

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IBRUTINIB IN THE MANAGEMENT OF RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS: A REAL-LIFE MONOCENTRIC EXPERIENCE

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Background: Ibrutinib is an irreversible inhibitor of Bruton tyrosine kinase showing significant efficacy in chronic lymphocytic leukemia (CLL) treatment. Routine healthcare may provide data complementary to that from clinical trials, in order to implement recommendations for disease management in real world settings. *Aim:* The aim of this retrospective study was to evaluate the outcome and toxicity of 25 CLL patients (pts) treated with Ibrutinib in real-life clinical practice in our Institution. *Methods:* Pts received ibrutinib 420 mg once-daily until progression or unacceptable toxicity. Overall response rate (ORR) including partial response (PR) with lymphocytosis (PR-L) was assessed using updated iwCLL criteria. *Results:* The median age was 70 years (range 54-93) with a male to female ratio of 2.1:1, 68% pts had Rai stage III/IV and 24% had lymphadenopathy ≥10cm. Pts received a median of 2 prior lines of therapy (range 1-6). The IGVH mutational status was unmutated in 13/25 pts and del(17p)/TP53 mutation was detected in 4/25 pts. After drug initiation, peripheral lymphocytosis was observed in 16 pts with peak levels at 1-month (median change from baseline in ALC was 191.2%±116.8-672.6%). In CLL with pre-existing cytopenias, a gradual improvement of anemia and thrombocytopenia was observed during treatment. ORR was 60% (32% PR, 20% PR-L, 8% CR). Out of 25 pts there were six with stable disease and four with disease progression or histological transformation. At a median follow up of 8 months, the median progression free survival (PFS) and overall survival (OS) have not yet been reached. The estimated PFS and OS rate at 8 months were 84% and 83% respectively. PFS and OS were significantly shorter in pts with del(17p)/TP53mut than in non-del(17p) patients (p=0.018 and p=0.026, log rank test). Ibrutinib was well tolerated. Common extra-hematologic AEs included infections (20%), diarrhea (16%) and skin lesions (8%). Hematologic AEs observed were neutropenia (12%), anemia (4%), thrombocytopenia (4%). Hematomas occurred in 16% of pts without any major bleeding. Grade of side effects was >3 in 6.6% of cases. The most frequent grade ≥3 AE was diarrhea (8%). Treatment was discontinued due to AEs in 2 pts and disease progression in 4 pts. Of all treated pts, 72% are still in treatment with Ibrutinib. *Conclusions:* Ibrutinib is highly effective and tolerable drug in CLL in general community. Yet, del(17p)/TP53 mutation remains a therapeutic challenge.

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ANALYSIS OF PROGNOSTIC FACTORS AND TIME TO FIRST TREATMENT IN YOUNG PATIENTS (<55 YEARS) WITH CHRONIC LYMPHOCYTIC LEUKEMIA: A SINGLE CENTER EXPERIENCE

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Chronic Lymphocytic Leukemia (CLL) is the most common leukemia among elderly people in the western world. Younger CLL patients (≤55 years) account for only 10% to 15% of the CLL population. Clinical characteristics and outcomes of Younger CLL patients are a matter of debate; a better knowledge is a crucial objective to identify patients with different prognostic features. In our study we investigated clinical characteristics and time to first treatment of younger patients with CLL treated at our Institution. We retrospectively evaluated patients (pts) with *de novo* CLL treated at the University Hospital of Bari (Italy) between April 2006 and February 2017.At diagnosis pts were studied for clinical characteristics and biological prognostic factors: age, -2 microglobulin, absolute lymphocyte count, sex, Rai stage, number of involved lymph node groups, pattern of bone marrow involvement, splenomegaly, mutational status of the immunoglobulin heavy chain gene variable region (IGVH). Patients were divided into two groups according to age: younger CLL pts (≤55 years) and older CLL pts (>55 years). Cytogenetic abnormalities at diagnosis were detected by FISH in 20 pts in the younger group and 35 pts in the older group. Time to first treatment (TFT) was measured as the time elapsed between diagnosis and first treatment.Patients were divided in two groups according to age. The first group included younger CLL pts (≤55 years); the second group included older CLL pts (>55 years). Univariate analyses were performed in each group to evaluate the correlation between age and clinical variables at diagnosis. TTF was also calculated and compared in the two groups. In total 75 pts were included; 24 pts under 55 years at diagnosis and 51 older pts at diagnosis. Statistically significant differences were seen between the 2 groups regarding absolute lymphocyte count (<50,000 *versus* >50,000 P<.001), -2 microglobulin (normal *versus* elevated; P=.009); splenomegaly (present *versus* absent; P=.035), cytogenetic abnormalities by FISH (P>.001), all significantly more common among older patients. By contrast, no statistically significant difference between the 2 groups was seen for TTF (P:0.139). In conclusion, although adverse prognostic markers were less common in our study among younger patients at diagnosis, the time to progression of younger pts in terms of TFT was similar to that in the older population. Multicentric studies and larger cohorts of patients are warranted to confirm these data.

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CHLORAMBUCIL PLUS RITUXIMAB AS FRONT-LINE THERAPY FOR ELDERLY PATIENTS WITH COMORBIDITIES AFFECTED BY WALDENSTROM MACROGLOBULINEMIA. A SINGLE CENTER EXPERIENCE

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Waldenstrom Macroglobulinemia (WM) is a rare B lymphoproliferative disorder characterized by an infiltration in bone marrow of lymphoplasmacytic lymphocytes producing an IgM monoclonal gammopathy. The standard first line treatment is Rituximab, Cyclophosphamide and Dexamethasone (DRC). We conducted a retrospective analysis of Chlorambucil-Rituximab (CHL-RTX) used as front-line treatment in elderly patients (≥65years) with comorbidities. CHL was administered at 1 mg/kg for each cycle every 28 days p.o. at a fixed daily dose of 10 mg starting from Day 1 and repeated for 8 cycles. RTX was added to CHL from the 3rd cycle onwards and was administered on Day 1 of each cycle at a dose of 375 mg/m^2 for 6 cycles.

Ten patients affected by WM were enrolled in this study, until December 2016. The patients required treatment for the onset of symptoms as anemia (6 patients), B symptoms such as weight loss (3 patients), peripheral neuropathy and hyperviscosity syndrome as loss of eyesight (one patient), respectively. The median number of CHL and RTX cycles administered was 8 (range 1-8) and 6 (range 3-6), respectively. The median total dose of CHL administered during treatment was 512 mg per patient and the median dose of RTX was 3600 mg per patient. In one patient, therapy was discontinued after 3 courses of CHL and 1 course of RTX because of the onset of severe pancytopenia and esophagus necrosis which led to the death of the patient. Two patients with low burden of paraprotein were able to avoid the two purging cycles of CHL, undergoing the 6 planned cycles of CHL-RTX. During the period under examination, none of the patients experienced a dose reduction of either CHL or RTX because of hematological/extra-hematological toxicities. ORR was 80%, one patient showed CR, one achieved a VGPR and six PR. Median PFS was reached after 31 months (range: 1-93 months) from the beginning of the treatment. All patients except for one, who died because of progressive disease during treatment, are alive at a median follow-up of 54 months. CHL-RTX was a very well tolerated regimen: only one patient developed grade 2 neutropenia without infective complications; no patient but one died of progressive disease and was admitted into hospital. The presented data showed that the association of CHL-RTX is safe and effective, CHL-RTX appeared as a good option as first line treatment in elderly patients with comorbidities for its measured balance between toxicity and response.

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CONTINUOUS LOW DOSE ALKYLANT THERAPY IS EFFECTIVE IN T-CELL PROLYMPHOCYTIC LEUKEMIA

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T-cell prolymphocytic leukemia (T-PLL) is a rare and aggressive mature T cell lymphoproliferative disorder, typically affecting elderly people, characterized by clonal expansion of CD4+ or CD4+/CD8+ lymphocyte cells with prolymphocyte morphology. Among cytogenetic abnormalities, recurrent alterations include inv(14) and t(X;14). Alemtuzumab represents the frontline therapy with median PFS and OS of 7 and 24 months, respectively. Alternative regimen like bendamustine showed PFS of few months. The aim of this study was to provide data on the efficacy and safety of a less intensive but continuous therapy in T-PLL patients not eligible to alemtuzumab therapy. We analyse a small cohort of 6 patients affected by T-PLL. Confirmation of diagnosis was achieved through combined morphologic, immunophenotypic and cytogenetic analysis. Clinical characteristics and therapy related parameters, including response, outcome and adverse effects were studied. Median age at the diagnosis was 72 years (range 63-85). All patients were characterized by CD4+/CD8 phenotype. Although a dominant V was reported in each patient, any clusterization to a specific V chain was detected. Cytogenetic analysis was available for 5 patients, with 2 patients presenting t(X;14), 2 patients with inv(14) and one patient with complex karyotype. HTLV antigens were not detected in any patient. At diagnosis, all patients presented lymphocytosis (18,000-75,000/mm3) with hepato-splenomegaly (3/6), diffuse lymphoadenopaty (4/6) and one patient with pulmonary interstitial disease. Five patients received continuous low dose cyclophosphamide (50-100 mg/die), one monthly chlorambucil chemotherapy. The ORR was 100% with 1 complete hematological response and 5 partial responses. Median PFS and OS were 15,5 and 20 months, respectively. Two patients died, one for progressive disease and one for severe sepsis. Hematological toxicities were mild with G2 neutropenia and G3 anemia observed in 2 patients. Non hematological toxicities were exclusively infectious events observed in 3/6 patients, with one lethal sepsis in old age patient. Our data provide evidence that low dose continuous alkylating therapy is effective and relatively safety in patients not eligible to intensive therapy. Although low quality of response, patients displayed similar PFS and OS rates to those treated with alemtuzumab and higher towards patients treated with other regimens.

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THE APOBEC MUTATIONAL ACTIVITY IN MULTIPLE MYELOMA: FROM DIAGNOSIS TO CELL LINES

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Next generation sequencing (NGS) studies have highlighted the role of aberrant activity of APOBEC DNA deaminases in generating the mutational repertoire of multiple myeloma (MM). However, the contribution of this mutational process across the landscape of plasma cell dyscrasias, or its prognostic role, has never been investigated in detail. To answer these unexplored aspects of MM biology, we used published NGS data from our own work as well as others, including the large CoMMpass trial for a total of 1153 whole-exomes of MM. Furthermore, we investigated 5 MGUS, 6 primary plasma cell leukemias (pPCL) and 18 MM cell lines (MMCL). Overall, we identified signatures of two mutational processes, one related to spontaneous deamination of methylated cytosines (30% of variants, range 0-100%) and one attributed to aberrant APOBEC activity (70% of variants, range 0-100%). APOBEC contribution was extremely heterogeneous among MM patients, but was correlated with a higher mutational burden (r=0.71, p=<0.0001) and with MAF gene translocations t(14;16) and t(14;20). The activity of APOBEC increased from MGUS to MM to pPCL, both in terms of absolute number of mutations and as percentage contribution. In MMCL we instead observed a bi-modal distribution whereby 8 cell lines showed the highest numbers of mutations caused by APOBEC (5/8 carried MAF translocations), while 10 where virtually devoid of APOBEC mutations (0/10 carried MAF translocations). The contribution of APOBEC to the total mutational repertoire in MM had a clear prognostic impact. MM patients with APOBEC mutations in the lowest quartile had a survival advantage over patients with APOBEC mutations in the highest quartile both in terms of progression-free survival (3-y PFS 46% *vs* 67% months, p=<0.0001) and overall survival (3-y OS 52% *vs* 83%, p=0.0084). This association was retained in a multivariate model that included age, gender, cytogenetic class, ISS, and quartiles of mutational load both in PFS [p=0.02, HR 2.06 (95IC 1.11-3.81] and OS [p=0.02, HR 2.88 (95IC 1.17-7.09)]. Interestingly we found that APOBEC mutations in the 4th quartile retained its independent prognostic respect to high mutational load and presence of MAF translocations. Overall, our data suggest that APOBEC-mediated mutagenesis is strongly involved in MM pathogenesis and its activity persists during different phases of evolution, playing a critical role in MM genomic complexity, and impacting prognosis of the patients.

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MULTIPLE MYELOMA BONE LYTIC LESIONS CHARACTERIZATION BY WB-LDCT SCAN AND 18F-FDG PET/MRI

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Multiple myeloma (MM) is a hematological malignancy characterized by clonal proliferation of plasma cells in bone marrow. Bone disease is the most frequent feature of MM and the major cause of comorbidity. IMWG settled that more than one focal lesion on MRI and at least one bone lytic lesion detected on whole body low dose CT (WB-LDCT) or 18F-FDG/TC fulfill the criteria for bone damage requiring therapy. The aim of this study was to correlate bone lytic lesions features evaluated by WB-LDCT to functional parameters detected by 18F-FDG PET/MRI imaging in newly diagnosed MM patients. We retrospectively studied 18 patients (12 males, 6 females), with median age of 57 years (range 42-73 years) affected by MM that at the time of diagnosis underwent both WB-LDCT and 18F-FDG PET/MRI. Only bone lytic lesions >5mm at WB-LDCT were considered and characterized for size and internal densitometry (negative or positive HU). The same lesions were evaluated with combined 18F-FDG PET/MRI registering T1w signal, T2w STIR (short tau inversion recovery) signal, DWI (diffusion weighted imaging) signal, mean ADC (apparent diffusion coefficient) value and SUV (standardized uptake volume) max value. By WB-LDCT 135 bone lytic lesions were recognized. 35 lesions had a negative densitometry (mean -57,57 HU; SD±33,13 HU) showing high signal in T1w images and low signal in STIR and DWI sequences. 100 lesions presented positive densitometry (mean 44,87 HU; SD±23,89) with low T1w signal and high signal in STIR and DWI sequences. Negative HU lesions presented significant lower sizes towards positive HU lesions (11.09 mm 0.75 *vs* 17.36 mm 1.32, p=0.0069). Interestingly, the first group presented lower ADC values (mean 360.69; SD±154.38) and lower SUV max values (mean 1.69; SD±0.56) as compared to the second group that showed higher ADC values (mean 868.46; SD±207.67) and SUV max values (mean 5.04; SD±1.94). Mean ADC values and mean SUV max values of the two groups resulted statistically different ($p < 0.0001$). Despite being a very sensitive technique to detect osteolytic lesions in MM patients, WB-LDCT scan is not appropriate to evaluate functional parameters as 18F-FDG or MRI do. Positive internal densitometry of osteolytic lesions would represent an additional parameter to point out bone destruction due to the presence of myeloma cells. 18F-FDG or MRI is particularly suitable for staging bone disease in patients presenting only low densitometry lesions at WB-LDCT scan.

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MINIMAL RESIDUAL DISEASE BY RQ-PCR AND MULTI-PARAMETER FLOW CYTOMETRY IN MULTIPLE MYELOMA: A POOLED ANALYSIS OF 2 PHASE III STUDIES IN PATIENTS TREATED WITH LENALIDOMIDE AFTER FRONT-LINE THERAPY

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Minimal residual disease (MRD) has emerged as one of the most relevant prognostic factors in multiple myeloma (MM) patients (pts). The role of maintenance therapy to modulate MRD levels is still unclear. The aim of this study is to evaluate the role of MRD by multi-parameter flow cytometry (MFC) and real-time quantitative PCR (RQ-PCR) as predictor of progression-free survival (PFS) in newly diagnosed MM (NDMM) pts receiving Lenalidomide maintenance. NDMM pts enrolled in the RV-MM-EMN-441 (NCT01091831) and the RV-MM-COOP-0556 (EMN02/HO95 MM) phase III trials achieving ≥ very good partial response after consolidation/intensification were included in the MRD analysis. All pts received Lenalidomide maintenance until progression. MRD analysis was performed on bone marrow (BM) after intensification/consolidation, after 6 courses of maintenance and then every 6 months until clinical relapse. Both RQ-PCR and MFC were employed. Molecular CR (m-CR) was defined by RQ-PCR with minimal sensitivity of 10 5. Immunophenotypic CR (flow-CR) was defined by MFC with a sensitivity of 10-4 and 10-5 respectively in RV-MM-EMN-441 and RV-MM-COOP-0556 protocols. MRD study included 73 pts with a median age of 57 years (37-65). After consolidation/intensification: 33/73 (45%) pts achieved m-CR and 44/70 (63%) pts achieved flow-CR. Median follow-up was 48.4 months and MRD was evaluated after consolidation: median PFS was 48.8 months *versus* not reached in no m-CR *vs* m-CR pts, respectively (p=0.003); median PFS was 41.5 months *versus* not reached in no flow-CR *vs* flow-CR pts, respectively (p<0.001). Among pts MRD positive after consolidation, 12/38 (31%) obtained a m-CR and 7/26 (27%) obtained a flow-CR during maintenance. In multivariable Cox analysis, the risk of progression/death was higher compared to standard risk factors both for pts who did not achieve m-CR during treatment *versus* pts who did (HR, 4.25 CI: 1.83-9.85, p=0.001) and for pts who did not achieve flow-CR during treatment *versus* pts who did (HR, 5.44 CI: 2.36-12.51, p<0.001). We identified a very high risk group defined by high risk FISH at diagnosis and persistent MRD positivity, with a median PFS of 29.4 months. MRD status, both with RQ-PCR and MFC, is a predictor of outcome significantly superior to standard risk factors in NDMM pts and the achievement of MRD negativity seems to overcome the high risk FISH status in PFS analysis. Lenalidomide maintenance further improved MRD negativity rate.

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CARDIOVASCULAR TOXICITIES IN TRANSPLANT INELIGIBLE PATIENTS WITH NEWLY-DIAGNOSED MYELOMA TREATED WITH CARFILZOMIB

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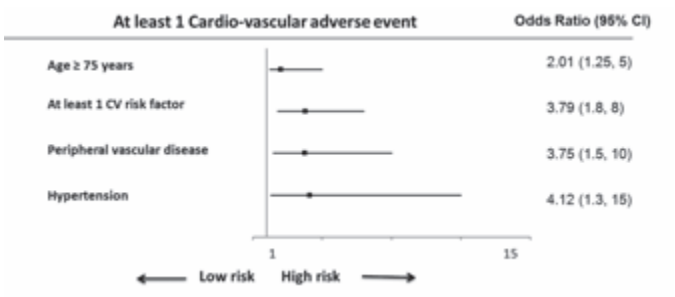
Background: Multiple myeloma (MM), comorbidities and MM treatment may cause cardio-vascular (CV) adverse events (AEs). Carfilzomib (K) is approved for relapsed MM. We conducted an integrated analysis of CV AEs in newly diagnosed, transplant-ineligible MM pts treated with K. *Methods:* All pts were treated with 9, 28-day induction cycles with K, cyclophosphamide (300 mg/m^2 on days 1,8,15) and dexamethasone (40 mg weekly) (CCyd), followed by K maintenance. K was given at 36 mg/m^2 d 1,2,8,9,15,16 in the IST-CAR-506 trial; at 3 dose levels escalated from 45 to 70 mg/m^2 d 1,8,15 in the IST-CAR-561 trial and on d 1,2,8,9,15,16 in the IST-CAR-601 trial. AEs were graded based on NCI-CTCAE v4.

Table 1.



Results: 148 pts were analyzed, median age was 72 years. 34% had at least 1 CV risk factor: 20% had peripheral vascular disease (including hypertension in 13% pts), 19% diabetes and 5% chronic pulmonary disease. After a median follow-up of 21 months, 45% had ≥1 any G CV-AE, 17% any G hypertension, 9% dyspnea, 6% heart failure, 6% arrhythmia and 6% venous thromboembolism (VTE). G 3-5 CV-AEs occurred in 15% of pts, heart failure (4%), hypertension (3%), pulmonary edema (3%) and VTE (3%) were the most common. Four (3%) fatal CV-AEs occurred: 1 heart failure, pulmonary edema, arrhythmia

and VTE, respectively. No difference in CV-AEs was observed with different doses of K. In pts who had ≥1CV-AE, K dose reduction (33%) and discontinuation (33%) were more frequent compared to those without CV-AEs (12% and 18%, p<0.0001). Pts who experienced ≥1CV-AE had a shorter 2-yr OS (adjusted for age) compared with those who did not (74% *vs* 83%, HR: 0.51; p=0.066). Pts ≥75 years had a higher risk of any G (58% *vs* 36%, p=0.02) and G 3-5CV-AEs (34% *vs* 15%, p=0.01); major cardiac events of any G were more frequent in older pts (29%) than in younger ones (6%; p<0.001). Pts with ≥1CV risk factor, hypertension or peripheral vascular disease had approximately 4-fold increased risk of developing CV-AE during treatment compared to pts with no CV risk factors: *Conclusions:* ≥1CV-AE occurred in 45% of pts, hypertension and dyspnea were the most common. Pts ≥75 years and those with ≥1pre-existing CV risk factor were at higher risk of developing CV-AEs. CV AEs significantly increased dose reductions and treatment discontinuation, with an impact on compliance and survival. All pts receiving K, particularly the elderly, should be carefully assessed.

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KRD (CARFILZOMIB PLUS LENALIDOMIDE PLUS DEXAMETHASONE) IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA IN CAMPANIA REGION: A ONE-YEAR SURVEY

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From April 2016, Carfilzomib, a second generation proteasome inhibitor, became available for use in routine practice in Italy. Approved indication is in combination with Lenalidomide and Dexamethasone (schedule according to Aspire trial) for patients with relapsed or refractory Multiple Myeloma (MM). We performed a survey in the whole Campania region to evaluate number and characteristics of patients treated accordingly so far. In 10 out of 11 Hematologic Divisions at least one patient has been treated with KRD. Most relevant clinical and disease data of the total 53 patients are summarized in the Table. Median number of courses administered so far is 3 (range 1-11). At the moment of writing, 45 cases are evaluable for response, with Overall Response Rate of 89%. We observed: Complete Remission=6 (median time to CR: 4 cycles), Very Good Partial Remission=20, Partial Remission=14. In five cases there was no response to treatment.

Table 1. Patients characteristics.

Number of patients	53
Sex: M/F	34/19
Median age at diagnosis, years (range)	58 (38-76)
Median age at KRD, years (range)	62 (42-81)
Number of previous lines:	
One	14 (26%)
Two	20 (38%)
Three	12 (23%)
≥ Four	7 (13%)
Previous ASCT: Y/N	41 (77%) / 12 (23%)
Previous Bortezomib: Y/N	51 (96%) / 2 (4%)
Previous Lenalidomide: Y/N	28 (53%) / 25 (47%)
Best response to previous treatments:	
Complete remission	18 (34%)
Very good partial remission	27 (51%)
Partial remission	7 (13%)
No response	1 (2%)
Median duration of PFS1, months (range)	29 (3-110)
Reason for treatment with KRD:	
Biochemical relapse	8 (15%)
Symptomatic relapse	23 (43%)
Refractory to last treatment	22 (42%)
Laboratory at KRD:	
Median WBC (x10E3/ul) (range)	4.87 (1.45-10.4)
Median Hb (g/dl)(range)	11.2 (7-16.4)
Median Plt (x10E3/ul) (range)	161 (16-383)
Median Creatinine Clearance (ml/min)(range)	98 (15-122)
Median LDH (IU/L)(range)	277 (107-551)

Two responding patients have relapsed after 4 and 9 months of Progression Free Survival (PFS). Six patients have died (1 relapsed and 4 refractory patients from progressive disease; 1 patient in PR from sepsis). Hematologic toxicity was negligible and present expecially during the first courses. Transfusion support was necessary in only 6 patients. Infections were recorded in 17 patients (1 fatal; 16 grade two or three, mainly bronchitis). In 12 patients at least one episode of arterial hypertension requiring treatment was observed. One patient with previous cardiopathy and diabetes suffered from acute myocardial infarction, one patient experienced atrial fibrillation, and in both cases treatment with K was discontinued. Finally, three cases of thrombosis and one case of tumor lysis syndrome were recorded. In 24/53 patients (45%) no toxicity at all was observed. Delay of treatment was necessary in 17 patients. Dose of K was reduced in 2 cases during treatment, while in 7 less fit patients a lower dose was used from the start. Medium number of admissions to Day Hospital Unit per single cycle was 6.7, which compares unfavorably to other regimens, namely RD. Hospitalization for treatment was needed in one patient, due to MM related poor PS. In conclusion, our data confirm that KRD is highly effective in relapsed refractory MM but needs careful evaluation of toxic effects, expecially cardiological. KRD has rapidly become a standard treatment for RRMM, but longer follow up is needed to further confirm its superiority to other regimens.

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LONG TERM CR MULTIPLE MYELOMA PATIENTS STUDIED WITH NEXT GENERATION FLOW SHOW PREDOMINANTLY CURED OR MGUS-LIKE MINIMAL RESIDUAL DISEASE PATTERNS

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In the era of novel agents, many multiple myeloma (MM) patients can achieve stringent CR (sCR), but most of these patients still will relapse and minimal residual disease (MRD) detection will play a crucial role in the very next future. Recently, two 8 colours tubes panel developed by the EuroFlow Consortium can detect MRD as standardized method to study MM patients.Little is known about long term remission patients (> 2-10 years) and MRD. Aim of the study was to analyze patients with MM in response > very good partial remission (VGPR) with NGF at > 2 and > 5 years of lasting remission after several treatments. 63 patients were studied of which 59/63 were evaluable. 59 samples of MM patients (M/F= 33/26) were studied with NGF between February 2016 and April 2017. Median age was 61 years (range 37-77 ys). 29/59 (49%) patients were in sCR at the moment of the study, 4/29 patients were under therapy, the others 25 sCR were evaluated at a median of 44 months after therapy (range 3-140).31/59 (52.5%) patients were in VGPR at study analysis according to new IMWG response criteria, 21/31 patients were off therapy from a median of 9 (range 2-186 months). N= 12, 26 and 47 patients had a remission disease >5 years (10/12 in sCR, only one patient under therapy, 2/12 in VGPR), >2 years (17/26 in sCR, 9/26 in VGPR, 3/26 under therapy) and <5 years (18/47 in sCR), respectively. Standard protocol was applied (OneFlow™ PCST and PCD, BD Biosciences) to detect MRD level with a lyse-wash-and-stain sample preparation protocol by flow cytometry (FACScanto II, BD, Biosciences). Discrimination between phenotypically aberrant (aPC) and normal PC (nPC) were carried out.MRD+ status was detected in 26/59(44%),3/12(25%) were MRD positive at >5 years remission (2 sCR, 1 VGPR) (median 84 months, range 72-186); 23/47 (48.9%) were positive at < 5 years of remission (5 CR;18VGPR)(median 9, range 2-56 months). 8/26 (34.6%) were MRD+after >2 years of remission (3 sCR, 6 VGPR)(median 48, range 28-186 months). As expected, being in sCR was correlated with a low MRD+ status 7/29 (24%) (2 patients after >5 years, 5 patients after <5 years). Interestingly looking at long lasting remission, *i.e.* >5 years, the 3/12 patients that resulted MRD+ displayed an MGUS like –plasmacell immunophenotype (prevalence of normal plasmacells *vs* aberrant monoclonal) with a PCn/PCtot ratio of 48%, 85%, 30%. CT/PET was positive in 7/59 patients. All patients in sCR were CT/PET negative. In conclusion NGF showed that MM patients with long remission status can be considered disease free/cured with a high sensitivity method.

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BONE MARROW DICKKOPF 1 (DKK-1) LEVELS ARE A NEW INDEPENDENT FACTOR FOR PROGRESSION IN PATIENTS WITH SMOLDERING MYELOMA

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New potential biomarkers to identify high risk smoldering multiple myeloma (SMM) need to be defined. Recently, we found that among the soluble factors involved in the bone remodeling, bone marrow (BM) levels of Activin A, C-C motif chemokine ligand 20 (CCL20), Dickkopf 1 (DKK-1) and osteoprotegerin (OPG) were significantly different among SMM and MM patients. Based on these evidences, we focused on those soluble factors in order to evaluate their possible role as possible markers of risk progression in SMM patients. We analyzed a total cohort of 89 patients with SMM as defined by the International Myeloma Working Group (IMWG) updated diagnostic criteria for MM and related disorders admitted to our Myeloma Unit between 2007 and 2015 and who underwent to BM aspirate. With a median follow-up of 34 months, 25 SMM patients subsequently progressed to active MM. We firstly analyzed the main clinical features at diagnosis, finding that BMPCs, serum M protein and immunoparesis were statistically significant correlated with progression to active MM ($p<0.0001$, $p<0.0001$ and $p=0.005$ by univariate Cox regression analysis, respectively). BM Activin A, CCL20 and OPG median levels were not significantly different between progressed and not SMM patients. Conversely SMM patients progressed to active MM showed significantly higher DKK-1 BM levels as compared to patients who had not progressed (median levels: 1267 *vs* 778.7 pg/mL, $p=0.001$). Interestingly, BM DKK-1 levels and percentage of BMPCs were not statistically significant correlated ($p=0.1117$). In multivariate analysis, adjusted for standard risk factors such as size of serum M protein,% of BMPCs, presence or absence of immunoparesis, we found that BM DKK-1 levels remained an independent prognostic factor for progression to active MM ($p=0.001$). Moreover, we performed a survival analysis stratifying patients according to BM DKK-1 levels finding that time to progression (TTP) was shorter in patients with higher levels of DKK1 as compared to patients with lower levels. Finally, ROC curve analysis showed that DKK-1 predicted the progression to symptomatic myeloma with a 78% specificity and a 60% sensitivity (AUC=0.677, CI=0.54-0.81). In conclusion, our study indicates that BM median levels of DKK-1 are able to identify a subset of SMM patients with higher risk of progression to active MM, and may represent a new independent risk factor for progression in SMM patients.

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LONG-TERM FOLLOW-UP AND IMPACT OF "NEW DRUGS" AT RELAPSE AFTER UPFRONT AUTO-ALLO TRANSPLANT IN MULTIPLE MYELOMA

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Prior to the introduction of "new drugs", we designed a multicenter trial where 162 newly diagnosed multiple myeloma patients were biologically randomized to receive either an autograft followed by a non-myeloablative allograft (N=80) or a double autograft (N=82). Fifty-eight/80 patients in the allograft arm and 46/82 in the double autograft arm eventually completed the assigned treatment. At a median follow-up of 12.2 (range 7.7-15.4+) years from second transplant, median overall survival (OS) was 11.4 in the allograft arm and 3.9 years in the autograft arm ($p=0.007$), whereas event free survival was 3.6 and 1.5 years ($p<0.001$), respectively. Two-year cumulative incidence of chronic graft-*versus*-host disease (GVHD) was 67.2%. At 5 years, 39% of the patients who developed chronic GVHD were alive, disease-free and off immuno-suppression (IS); 12% had died 37% had relapsed and 12% were still on IS. In both arms, main reason for treatment failure was disease recurrence. Overall, 33/58 patients (allograft arm) and 39/46 (autograft arm) relapsed at least once and were rescued with "new drugs" including thalidomide, lenalidomide or bortezomib. Specifically, at follow up, in the allograft arm, two patients had experienced biochemical relapse without reaching criteria for re-treatment and were alive at 11 and 13 years from diagnosis; overall 28/33 were rescued with new drugs in different combinations. The remaining patients received either donor lymphocyte infusion alone or conventional chemotherapy. Two patients eventually underwent a second allograft. In the autograft arm, 35/39 patients were rescued with new drugs. Salvage treatments also included a third autograft in 13 and an allograft in 3. Of note, median OS from 1st relapse was 7.5 years in the allograft arm and 2 years in the autograft arm (HR 0.47, CI 95% 0.26-0.84; $p=0.01$). In summary, long-term disease free survivors were significantly more frequent in the allograft arm. At relapse, response rates to "new drugs" were significantly better in the allograft arm. Given that MM remains an incurable disease despite a dramatic improvement in survival outcomes in the past decade, the combination of new drugs and allografting should be prospectively explored in high risk patients where prognosis remains poor also in the era of new drugs.

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TREATMENT OPTIMIZATION FOR MULTIPLE MYELOMA: SCHEDULE-DEPENDENT SYNERGISTIC CYTOTOXICITY OF POMALIDOMIDE AND CARFILZOMIB ON AN IN VITRO AND EX-VIVO MODEL

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Despite recent advances in treatment, Multiple Myeloma (MM) remains an incurable disease for the majority of patients. To improve the outcome, novel targeted therapies and synergistic combinations with appropriate anti-MM agents are needed. Immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs) form the backbone of modern MM treatment. In the present study, we examined the effects of different combinations schedule using second-generation PI (Carfilzomib - CAR) and third-generation IMiD (Pomalidomide - POM) (*i.e.* simultaneous exposure of POM and CAR, sequential drug combo in which cells were treated with CAR for 10h followed by POM or POM for 10h followed by CAR). Apoptosis analysis was done up to 48h after administration of the first drug. For each drug, three different concentrations were used: low dose, intermediate dose and high dose. In order to differentiate the complex interaction between BMSCs and plasma cells, we used three different experimental conditions: 1) MM cells cultured in complete medium, 2) MM cells suspended in medium conditioned in the prior presence of BMSCs, or 3) MM cells co-cultured with BMSCs in a transwell system. For the purpose of the study, we used five bona fide MM cell lines, a human bone marrow stromal (BMS) cell line and primary samples from newly diagnosed MM patients. Using the median effect method of Chou Talalay, we evaluated the combination indices for simultaneous and sequential treatment schedules, and we found that the schedule of administration is important to maximize the synergistic effects. Indeed, schedule-dependent synergistic cytotoxicity was demonstrated for the combination of POM and CAR and a maximal apoptosis consistently observed in POM pre-exposure schedule. The superiority of this schedule was maintained throughout BM microenvironment models using low dosage of both drugs. Our data overall suggest that the administration of POM before CAR can improve efficacy. Clinical trials are needed to investigate the most effective schedule, which could be to start the administration of IMiDs a day before PIs to increase cells killing. Whilst the clinical efficacy of CAR and POM combinations has been demonstrated, the synergistic cytotoxicity may be further exploited by using optimized schedule. Utilizing such

a schedule with IMiDs pre-treatment may improve the depth and duration of response of MM patients both as upfront therapy and in the relapsed/refractory setting.

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FLOW CYTOMETRY REMISSION BY IG LIGHT CHAINS RATIO ON PLASMA CELL SUBPOPULATIONS PREDICTS PROGRESSION-FREE SURVIVAL IN ELDERLY MULTIPLE MYELOMA PATIENTS TREATED WITH VMP

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Flow Cytometry (FC) is a powerful technique in both diagnosis and Minimal Residual Disease (MRD) identification in multiple myeloma (MM) patients. However, most of data were obtained into controlled clinical trials and if MRD assessment by FC is routinely applicable to clinical practice is so far under consideration. From January 2011 to January 2015, 37 consecutive MM patients, median age 73 years (57-83) treated with a program containing Bortezomib, Melphalan and Prednisone (VMP) for a total of 9 courses entered the study. Plasma-cells (PC) FC characterization was performed on bone marrow (BM) samples using a 6-colors panel of antibodies (Fitc/PE/PerCP/PE-Cy7/APC/APC-Cy7) evaluating the cy-Ig lambda/cy-Ig kappa light chains expression on CD19 CD38 CD56/CD117 CD45 surface markers, for a total of 19 kappa/lambda ratio evaluations. BM B lymphocytes were used as internal control for cy-Ig light chains expression. The analysis was performed at diagnosis and after 30-60 days from the end of the last course of treatment for flow-MRD assessment. Patients were considered flow-MRD negative when pathological PC were undetected in the BM samples at the FC sensitivity limit of 10-5 cells. A mean of 15420 PC was analyzed for MRD assessment, allowing a maximum sensitivity of detection of 0.0001% in all cases. At the end of VMP, 18 patients (48%) achieved a standard CR, 10 a VGPR, 3 a PR and the remaining 6 a SD/PD (three of these did not complete the treatment for progression of disease). Among 18 patients in CR, 12 (67%) were flow MRD-negative and 6 (33%) flow MRD-positive. Overall, MRD negativity by FC assessment is the better marker of clinical outcome in terms of PFS (2y-PFS: 90% *vs* 52% for flow MRD negative and positive, respectively; P=0.003). Moreover, among patients in standard CR, 2y-PFS of flow-MRD negative patients was significantly better than MRD positive (P=0.012). As previously documented on MM patient after autologous hematopoietic stem cell transplant, FC remission through cy-Ig light ratio on PC subpopulations is a low-cost, sensitive and applicable MRD assay, a powerful tool in treatment response evaluation and a crucial marker of outcome in MM.

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A NOVEL IN-HOUSE DEEP-SEQUENCING METHOD FOR NON-INVASIVE DISEASE MONITORING IN MULTIPLE MYELOMA PATIENTS

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Background: Novel and more effective treatment strategies have significantly prolonged multiple myeloma (MM) survival and raised interest in the depth of response. This implies the need of highly sensitive assays such as the determination of minimal residual disease (MRD) by multiparametric flow cytometry (MFC) and next generation sequencing (NGS) of immunoglobulin (IGH) gene rearrangements. Ongoing studies are examining circulating cell-free tumor DNA (cfDNA) as a sensitive measure of small amounts of residual cells. In the present study, we describe and analytically validate a simplified in-house deep-sequencing method to identify and quantify residual tumor burden in MM patients from plasma samples. *Methods:* We retrospectively analyzed 25 MM paired tumor (n=25) and plasma samples (n=48) obtained at diagnosis and at specified time points during treatment. Genomic DNA (gDNA) and cfDNA were extracted from selected CD138+ plasma cells (PC) and from plasma (Qiagen). IGH gene rearrangements were amplified, quality assessed (Agilent hsDNA kit) and sequenced on Ion Torrent PGM. Raw reads were filtered and aligned using IMGT germline database and aggregated into clonotypes. Post-processing analyses were performed using VDJtools and customized R scripts. *Results:* Our sequencing method successfully identified a IGH MM clonotype in 88% of tumor samples (22/25), subsequently detected in plasma of all 22 cases (median 4.7% of total filtered reads). Levels of the IGH clonotype in cfDNA distinguished between groups of patients with different prognosis: patients with levels >4.7% prior to therapy, had significantly shorter PFS than patients with levels<4.7% (median 268 *vs* 990 days; HR=3.532, P=0.04827, Log-rank). IGH cfDNA levels over the median were significantly associated with higher risk of disease progression (HR=7.9, P=0.0384). cfDNA levels reflected the number of PC enumerated by MFC (r=0.7249, P<0.0001, Pearson's correlation test). Accordingly, TTP was significantly longer (P<0.0001) for patients displaying frequencies lower than 10-5 (TTP 9±3 months, mean±SD, for frequencies>10-5 *vs* 15±5 months for frequencies=10-5 *vs* 37±4 months for frequencies<10-5). Those patients are in CR and characterized by PC frequencies <10-5 by MFC, and are therefore defined as MRD-negative. *Conclusions:* Results of this study support the clinical applicability of quantifying tumor levels by our in-house deep-sequencing of IGH gene rearrangements in plasma of MM patients.

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PTOLOMAIC PROJECT: A LOOK ON MYELOMA BEYOND THE DISEASE

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Aim: To outline a picture of the world of patients (pts) with myeloma and their caregivers (cgs) trying to identify their unmet needs and to explore how they face this distressing experience inside and outside the hospital. *Methods:* Pts and cgs filled in some questionnaires regarding their satisfaction, at the time of enrolment and after 6 months (t0 and t1). Pts were also evaluated for their fitness with: non-instrumental and Instrumental Activities of Daily Living (ADL and IADL), Study of Osteoporotic Fractures (SOF), Geriatric Depression Scale (GDS) and Cumulative Illness Rating Scale (CIRS). Cgs were evaluated with Caregiver Burden Scale (CBS). One hundred seventy two pts were included in the study,136 of which (79.1%) had a cgs. *Results:* Features of pts and cgs at t0 are reported in Table1. A quarter of pts were at disease onset (≤1year), 28% were on treatment. Basing on the questionnaires,in case of emergency about half of pts ask for help firstly to cgs, while most of cgs ask general practitioner. Median (mdn) satisfaction level for global hospital service was high in both groups. Mdn cgs burden scale was 10. Forty-six pts (27%) were defined depressed (GDS≥2). Most of pts reached showed a high score for ADL and IADL and IADL was higher in males *vs* females (85% *vs* 70%,p=0.025). Eighty-nine pts (52%) were defined frail (at least one SOF criteria) and these were older than the others (67 *vs* 63 years,p=0.020). Mdn score for psychiatric pathologies, severity and comorbidity were 2, 0.07 and 0. No difference in ADL, IADL, SOF scales and in severity and comorbidity indexes were found according to the duration and the phase of disease at t0 (all p>0.1). After 6 months,135 pts (78.5%) were evaluated again. Percentage of frail pts decreased among pts with a duration of disease >1year (t0:48% t1:33%,p=0.009) and among pts treatment-free at t0 (t0:47% t1:29%,p=0.004). Mdn severity and comorbidity indexes decreased (p<0.001) with no difference according to the duration of disease and to the disease phase at t0 (all p>0.4). Mdn score for psychiatric pathologies decreased in the whole cohort (t0:2 t1:0,p=0.012), in pts at disease onset (t0:2 t1:0,p=0.006) and in pts treatment-free at t0 (t0:1 t1:0,p=0.043). ADL and IADL did not change (p=0.5 and p=0.8). *Conclusions:* When dealing with a complex disease as myeloma, it is important to extent the health professional intervention to an accurate evaluation of the emotional and practical difficulties of people (pts and cgs) who have to face such a distressing experience.

Table 1. Features of patients and caregivers at the enrolment.

	Patients (n=172)	Caregivers (n=136)
Age in years, median (range)	65 (38-86)	58 (23-84)
Sex, n (%)		
Males	94 (54.7%)	52 (38.2%)
Females	78 (45.3%)	84 (61.8%)
Marital Status, n (%)		
Single	8 (4.7%)	11 (8.3%)
Married	138 (80.2%)	116 (87.2%)
Divorced/Separated	10 (5.8%)	5 (3.8%)
Widower	16 (9.3%)	1 (0.7%)
Number of sons, n (%)		
Childless	21 (12.2%)	22 (16.6%)
1	54 (31.4%)	45 (33.8%)
2	73 (42.4%)	51 (38.3%)
≥3	24 (14.0%)	15 (11.3%)
Qualification, n (%)		
Primary school certificate	43 (25.0%)	19 (15.6%)
Middle school diploma	43 (25.0%)	31 (25.4%)
High school diploma	67 (38.9%)	61 (50.0%)
University degree	19 (11.1%)	11 (9.0%)
Occupation, n (%)		
Housewife	10 (5.8%)	14 (11.4%)
Unemployed	4 (2.3%)	5 (4.1%)
Office worker	17 (9.9%)	16 (13.0%)
Freelance / Entrepreneur	10 (5.8%)	10 (8.1%)
Workman	5 (2.9%)	4 (3.2%)
Retired	109 (63.4%)	53 (43.1%)
Other	17 (9.9%)	21 (17.1%)
Degree of kinship with patient, n (%)		
Spouse	-	94 (69.1%)
Son / Daughter	-	3 (2.2%)
Parent	-	27 (19.9%)
Other relative	-	12 (8.8%)
Home-Hospital Distance, n (%)		
<10 Km	20 (11.6%)	-
10-100 Km	125 (72.7%)	-
>100 Km	27 (15.7%)	-
Disease duration (years), median (range)	4 (0-28)	
≤1 year	44 (25.6%)	-
>1 year	128 (74.4%)	-
Disease phase, n (%)		
On treatment	38 (28.2%)	-
Treatment-free	97 (71.8%)	-
ADL (maximum score), n (%)	154 (89.5%)	-
IADL (maximum score), n (%)	134 (78.4%)	-
Frailty (at least one SOF criteria), n (%)	89 (51.7%)	-
CIRS Severity index, median (range)	0.07 (0-1.43)	-
CIRS Comorbidity index, median (range)	0 (0-5)	-
CIRS Psychiatric pathologies score, median (range)	2 (0-4)	-
Depression (GDS score≥2), n (%)	46 (27.2%)	-
Global caregiver burden scale, median (range)	-	10 (0-55)
Objective burden, median (range)	-	3 (0-20)
Evolutionary burden, median (range)	-	2 (0-18)
Social burden, median (range)	-	1 (0-11)
Physical burden, median (range)	-	2 (0-16)
Emotional burden, median (range)	-	0 (0-6)
Satisfaction level of global hospital service, median (range)	9 (5-10)	9 (4-10)
Reference contact if necessary, n (%)		
Relative	85 (49.4%)	27 (23.3%)
General Practitioner	70 (40.7%)	70 (60.3%)
Hospital	17 (9.9%)	19 (16.4%)

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TREATMENT PATTERNS AND OUTCOMES IN BIOCHEMICAL AND CLINICAL RELAPSE: RESULTS FROM THE ITALIAN POPULATION OF THE ONGOING, OBSERVATIONAL PREAMBLE STUDY IN MULTIPLE MYELOMA

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Background: Patients (pts) with multiple myeloma (MM) eventually develop asymptomatic, biochemically relapsed disease (BRD), or symptomatic, clinically relapsed disease (CRD) that includes hypercalcemia, renal insufficiency, anemia and bone lesions (CRAB features). This analysis of the ongoing, observational, international, PREAMBLE cohort study (NCT01838512) examined treatment patterns and outcomes for pts enrolled in Italy, to provide insight into optimal treatment strategies for pts with BRD or CRD. *Methods:* For this analysis, pts (age ≥18 y, with relapsed/refractory MM, ≥1 prior therapy) initiated treatment ('index therapy') with an immunomodulatory drug (IMiD) or proteasome inhibitor (PI) from 90 d prior to 30 d after study consent. BRD was defined as 25% increase from lowest M protein response value, free light chain ratio, or bone marrow plasma cell percentage; CRD as development/worsening of CRAB features. *Results:* At data cut-off (Sept 2016), 215 pts from Italy had been treated; median (Q1–Q3) follow-up was 17 (11–25) mo. Of 176 pts evaluable by relapse type, 68 (39%) had BRD and 108 (61%) had CRD. At baseline, greater proportions of pts with CRD *vs* BRD had refractory MM and prior transplantation. Of pts with BRD, 51% received IMiD index therapy and 47% a PI; 48% with CRD received an IMiD and 51% a PI. The most common prior regimen was bortezomib+dexamethasone (21% pts with BRD; 25% CRD). In pts with CRD, PI *vs* IMiD index therapy was more frequent in pts with no comorbidities, >1 prior line of therapy and prior transplantation (Table). Median PFS was greater with BRD *vs* CRD, and for pts who received an IMiD *vs* PI for both relapse types. Pts with PI index therapy had longer time from diagnosis to treatment, regardless of relapse type (Table). Duration of index therapy was longer in pts receiving IMiD *vs* PI, and with BRD *vs* CRD; duration of last prior therapy was similar between groups. Response rate (≥MR) was greater in pts with BRD *vs* CRD, and similar for IMiD *vs* PI in pts with BRD; pts with CRD had a higher response rate with IMiDs *vs* PIs. Response rates at 6 and 12 mo were greater for BRD *vs* CRD, and for pts with CRD who received an IMiD *vs* PI. Median time in response is shown in the Table. *Conclusions:* In Italy, outcomes were improved for pts starting index therapy with BRD *vs* CRD, and who received continuous IMiD index therapy *vs* fixed PI therapy; however, pt numbers are small and results should be interpreted with caution. Study support: BMS.

Table 1.

Table. Baseline characteristics and treatment outcomes for patients in PREAMBLE with available data enrolled and treated from Italy

	All pts from Italy (N=215)	BRD (n=68)[a]		CRD (n=108)[a]	
		IMiD (n=35)	PI (n=32)	IMiD (n=52)	PI (n=55)
Age, median (range), y	70 (42–86)	71 (42–83)	70 (43–83)	70 (51–86)	66 (45–84)
35–64	71 (33)	12 (34)	8 (25)	17 (33)	23 (42)
65–74	88 (41)	11 (31)	16 (50)	23 (44)	21 (38)
≥75	56 (26)	12 (34)	8 (25)	12 (23)	11 (20)
Male	122 (57)	18 (51)	16 (50)	32 (62)	37 (67)
Relapsed MM	162/214 (76)	30 (86)	28 (88)	35/51 (69)	38 (69)
Refractory MM	52/214 (24)	5 (14)	4 (13)	16/51 (31)	17 (31)
Comorbidities					
0	57 (27)	8 (23)	9 (28)	10 (19)	23 (42)
1–3	126 (59)	22 (63)	18 (56)	33 (63)	28 (51)
≥4	32 (15)	5 (14)	5 (16)	9 (17)	4 (7)
Prior LoTs					
Median (range)	1 (1–7)	1 (1–3)	1 (1–3)	1 (1–4)	2 (1–6)
1	117 (54)	19 (54)	19 (59)	29 (56)	25 (45)
>1	98 (46)	16 (46)	13 (41)	23 (44)	30 (55)
Prior transplantation	94 (44)	14 (40)	14 (44)	18 (35)	31 (56)
International Staging System stage III	33/151 (22)	7/21 (33)	2/24 (8)	8/37 (22)	11/42 (26)
Baseline cytogenetic abnormalities[b]	13/19 (68)	NE[c]	NE[c]	5/5 (100)	3/5 (60)
PFS, median (95% CI), mo	13 (11–15)	18 (12–NR)	13 (6–16)	12 (8–NR)	8 (5–14)
Achieved MR or better	88/149 (59)	21/30 (70)	18/25 (72)	21/35 (60)	16/38 (42)
6 mo rate, % (95% CI)	37 (30–45)	46 (29–62)	57 (38–76)	39 (24–55)	20 (8–32)
12 mo rate, % (95% CI)	55 (47–63)	66 (49–83)	69 (51–87)	53 (37–69)	42 (26–58)
Time in response, median (95% CI), mo	15 (10–NR)	NR (9–NR)	13 (8–NR)	16 (9–NR)	15 (3–NR)
Diagnosis to index therapy, median (range), mo	n=214 39 (3–205)	30 (7–161)	50 (6–205)	n=51 33 (3–156)	52 (4–160)
DoT of index therapy, median (range), mo	9 (1–38)	14 (1–38)	5 (1–24)	10 (1–38)	4 (1–27)
DoT of last prior LoT, median (range), mo	n=212 8 (0–71)	9 (0–30)	9 (2–50)	n=51 8 (1–35)	8 (0–71)

Data presented as n (%) unless indicated otherwise. [a]1 patient with each relapse type received an IMiD + PI combination; [b]Includes high- (del(17p) and t(4;14)) and standard-risk abnormalities; [c]Not evaluable (<4 evaluable patients). CI, confidence interval; DoT, duration of therapy; LoT, line of therapy; MR, minimal response; NR, not reached; PFS, progression-free survival

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LONG TERM TREATMENT WITH LENALIDOMIDE-DEXAMETHASONE FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Lenalidomide in combination with dexamethasone (len/dex) is approved in patients (pts) with relapsed/refractory Multiple Myeloma (RRMM) who have received at least one prior therapy. However, the toxicity profile of long-term exposure to len/dex and its impact on outcomes have not been well defined. We conducted a retrospective observational study to evaluate the clinical outcomes of RRMM pts who received len/dex therapy for more than 2 years. A total of 103 pts were included in the present analysis. The median age was 69 years and 64% of pts were male. The frequency of ISS stage 2 and 3 was 23% and 19%, respectively. The median time from diagnosis to start of len/dex was 3 years. The median number of prior lines of therapy was 1 (range 1-5). 43% of pts underwent autologous stem cell transplantation, while 45% and 77% were previously treated with thalidomide and bortezomib-based regimens, respectively. Overall, pts received len/dex treatment (doses adjusted for renal function and age) with adequate thromboprophylaxis for a median of forty-six 28-day cycles. 86% of pts achieved a best partial response (PR) or better (including 19% ≥CR and 41% VGPR) within a median time of 11 months. At the time of cut-off analysis, 60 pts (58%) discontinued treatment, 57% of these for disease progression and 13% for toxicity. Len dose reduction was reported in 74 pts (72%) after a median of 11 cycles. Dex dose adjustement was reported in approximately 60% of pts, with a definitive discontinuation in 54% after a median of 29 cycles. With a median follow up 50.9 months, median progression free survival (PFS) was 45.2 months. No significant differences in 4-year PFS estimates were observed according to len dose reduction (66.3% *vs* 64.6%, p=0.37) or dex discontinuation (70.4% *vs* 64.9%, p=0.43). Most frequently reported toxicities were neutropenia (58%), which proved manageable with appropriate len dose-reduction and G-CSF treatment, diarrhea (23%) and deep vein thrombosis (11%). 3 cases of second primary malignancies were reported at a median time of 25,3 months from start of therapy. Long-term treatment with len/dex is feasible in RRMM pts, with a well manageable toxicity profile. Approximately 11-12 cycles of len-dex therapy at optimal doses should be planned to maximize the depth of response. Len dose reduction and/or dex discontinuation do not adversely affect PFS after a plateau phase is achieved, and may allow an improved pt compliance to prolonged therapy.

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ASSESSEMENT OF MINIMAL RESIDUAL DISEASE BY MULTIPARAMETER FLOW CYTOMETRY IN PATIENTS WITH AL AMYLOIDOSIS

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Introduction: In multiple myeloma, Minimal Residual Disease (MRD) demonstrated by multiparameter flow cytometry (MFC) has a central role in the evaluation of response. In this study, we assessed MRD by MFC in patients with AL amyloidosis who attained complete response (CR). *Methods:* CR was defined as per current criteria (negative serum and urine immunofixation and normal free light chain ratio). For flow cytometry studies bone marrow samples were processed following the EuroFlow Bulk Lysis Standard Operating Protocol, stained with EuroFlowIMF MM MRD panel and analyzed with Infinicyt software. At least 5x106 events were measured using a FACSCanto II instrument. Patients were identified as having residual disease if a discreet population of clonal plasma cells comprising ≥50 events was identified (10-5 limit of detection). *Results:* Thirty-three patients were tested (8 were found to have relapsed at the time of MRD assessment with monoclonal components detectable and MRD+) and 25 satisfied current criteria for CR. At diagnosis, 22 (88%) had kidney and 11 (44%) had cardiac involvement with 4 patients in Mayo stage 3. More than 2 lines of therapy were required to achieve CR in 7 subjects. Median time to CR was 10 months (range: 3-83). Six out of 9 patients (66%) had achieved cardiac response and 11/21 (66%) renal response at the time of CR. Flow cytometry identified MRD in 10 patients (40%). The median time from CR to MRD was 30.8 months (range: 5-148), this was not different in the MRD positive *vs* negative patients. A median of 1168 (range 252-2500) corresponding to 0.04% (range 0.01-0.3%) plasma cells with abnormal phenotype were detected in patients MRD+. No differences in the principal clinical variables, type and number of treatments, and organ response at the time of CR were found between the two groups. However, a further improvement of cardiac function compared to the time of CR was observed in all 5 evaluable MRD- patients and in none of the 2 MRD+ patients (P=0.047). Compared to the time of CR, renal response was obtained in 9 MRD- subjects (81%) and in 4 (50%) MRD+ (P=0.094). Overall, further improvement of cardiac or renal function after CR was significantly associated with absence of MRD (P=0.002). *Conclusions:* In this unselected cohort, 40% of patients satisfying current criteria for CR have detectable MRD. MRD positivity is associated with a persistence of organ damage is patients in CR. A validation study in a larger cohort is ongoing.

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EXTRAMEDULLARY DISEASE SOFT-TISSUE OR BONE-RELATED IN MULTIPLE MYELOMA: CLINICAL FEATURES, BIOLOGICAL BEHAVIOR AND OUTCOME. SINGLE-CENTER EXPERIENCE OF 100 CASES IN THE ERA OF NOVEL THERAPY

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Extramedullary-disease(EMD) is an uncommon manifestation and seems to have a different pathogenesis from medullary-counterpart. There are concerns about increase of EM-relapses with use of biological-agents. We retrospectively-reviewed 100 myeloma-patients with detectable-extramedullary-plasmacytoma consecutively-diagnosed between 1999-2016. We included 50 patients presenting bone-related extramedullary disease (b-EMD)and 50 patients with soft-tissue related-EMD (s-EMD) comparing clinical-features and outcome. 41 among sEMD were dead and 9 were alive, 12-bEMD patients were dead and 38 were still alive. Of the first-group 10 presented EMD at diagnosis and 40 at relapse as well as 5 and 45 respectevely of the second-series. Among sEM group we described skin-involvement (20 patients), parenchyma (lung, breast, liver in 15), lymh-nodes (10) and central-nervous-system (CNS) in 5 patients.We showed that sEMD occurred more frequently in male-patients (40/50) and had higher-levels of B2microglobulin and lactate-dehydrogenase (LDH) (43/50) and high frequency of advanced-disease according to International-Staging-System(ISS) (III-stadium in 39patients, II in 8patients,I in 3patients). In the bEMD group they were 35females, 15 males, 11 had high-LDH or B2microglobulin-levels and majority of them has intermedium-ISS-score(36 I, 9 II,5 III). Compared to patients with bEMD, sEMD-patients had worse global median-overall-survival (30 months *versus* 48, P<0,0001-HR-1,6-95%CI-1,03-2,47). Similar results were obtained about median-OS from EMD-diagnosis (10 months *versus* 30, P<0,0001-HR-3-95%-CI-1,93-4,64). In s-EMD-group the worst prognosis belongs to CNS-involvement(median OS from EM-diagnosis 4-months), followed by parenchyma-EM(OS median 7) and by lymph-nodes-EM (median-OS 20)and lastly by skin-EM(median-OS-26-months) P<0,0001. Extramedullary-spread can be triggered by invasive-procedures. We had a case of breast-plasmocytoma diagnosed accidentally after reconstructive-breast-surgery.It has been described association between EMD, IgD-subtype and FLC-escape. We reported 6 cases of IgD and 6FLC-escape, all of them in relapse-setting and in sEMD-group. Extramedullary-spread are poorly understood: maybe a decrease-expression of integrins is involved. Absence of CD56-protein was shown in 66% of sEMD-group and in 19% of bEMD-case-series. Outcome of bEMD was significantly better than sEMD,and was comparable to the patients without EMD. Even in the "novel-agents-era" sEMD has a poor prognosis. We demonstrate a significant difference in prognosis for different-type of sEMD,suggesting a different biological-behavior.

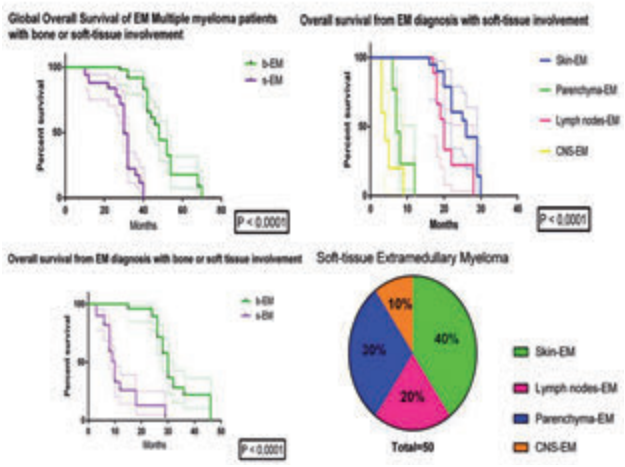

Figure 1.

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INFECTIONS IN MULTIPLE MYELOMA UNDER TREATMENT AS ONE OF THE MAJOR CAUSE OF MORBIDITY AND MORTALITY IN THE "NOVEL AGENTS ERA". A REAL-LIFE RETROSPECTIVE MONOCENTRIC EXPERIENCE

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New-treatments-options have improved survival of multiple-myeloma-patients. Effect of novel-therapies on the risk of infections remains to be established.We aimed to study development of severe-infectious-events of grade-3-4-in a group of patients under-treatment at diagnosis-and-at-relapse. We retrospectively reviewed 175myeloma-patients diagnosed from 1999-2016 to assess type and outcome of-infections. 7were-life-threatening (3viral-interstitial-pneumonias, 4gram-negative-sepsis), 97have resulted-in-therapy-discontinuation (69-bacterial, 10-fungal, 16-viral, 2-parasitic-infectious-complications). We analysed time of occurrence and number of prior-therapeutic-lines and disease-biological-aggressiveness. We aimed to define risk-factors and to organize an effective-antimicrobial-prophylaxis. 25 patients presented Fever-of-unknown-origin without-isolation of pathogens. 150 patients presented infectious-complications with well-known-etiology: 90 bacterial, 40 viral,15fungal and 5parasitic-infections. Pathogens-were: Candida-Albicans, parapsylosis among fungal-infections, E.Coli, Klebsiella between bacterial-complications, CMV, HSV in viral-manifestations and Leishmanias-among-parasitic-events. Advanced-age is a meaningful-risk-factor togheter with biologically-aggressiveness and relapse-condition. The majority of patients were older than 65 years (74%) in relapse-setting(72%). Kind of anti-myeloma-therapy used also plays a role in this setting. Predominant-part of patients-developing fungal-infections (86%) showed neutropenia after- chemotherapy or previous-therapy with Imids. The majority of patients with viral-infections (82%) presented-lymphopenia and previous-therapies-bortezomib-based. Bacterial-infections have shown mostly prevalent in neutropenic-phases (82%) usually in relapse-phases (70%) or in hypogammaglobulinemic-patients (68%). Most of parasitic-infections-are-described-after-high-doses-steroid-treatment and with more than 2-therapeutic-lines(100%). Infections represent a significant-comorbidity expecially in refractory/relapsed-patients. Immunoglobulin-replacement-therapy or antibiotic-prophylaxis may have a protective-role in old-patients with-high-ISS-stage. Variety of factors underlies susceptibility to infections including defect of innate and adaptive-immunity. Based-on-our-experience,in the first 3months-of-IMIDs- therapy we begin prophylactic-antibacterial and antifungal-therapy (quinolone-fluconazole). All patients treated to date(30-in-total) did not require therapy-discontinuation. High-risk-patients-should-receive-antimicrobial-prophylaxis and trials-on prophylactic-measures-are-needed.

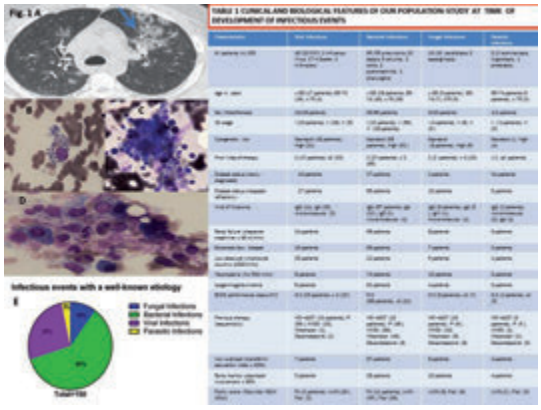

Figure 1.

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"REAL-LIFE" EXPERIENCE OF CARFILZOMIB COMBINED WITH LENALIDOMIDE AND DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS

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Background: Carfilzomib, a new proteasome inhibitor, was recently approved in Italy in association with lenalidomide and dexamethasone (Rd) for relapsed/refractory multiple myeloma (RRMM) patients (pts). Results from clinical trials ENDEAVOR and ASPIRE have shown an improved outcome in carfilzomib treated RRMM pts but have also warned the clinicians against a possible risk of cardiac toxicity. *Methods:* of the study: The aim of this retrospective single centre study was to evaluate the efficacy and tolerability of carfilzomib in association with Rd (KRd) in RRMM pts registered within the Kyprolis Post Approval Access Protocol (PAAP) or, subsequently, within the Agenzia Italiana del Farmaco (AIFA) registry. Each patient underwent cardiologic evaluation including echocardiogram and EKG before starting therapy. Carfilzomib was administered intravenously on days 1,2,8,9,15 and 16 (20 mg/mq on days 1 and 2 of cycle 1 then 27 mg/mq) for 12 28-days cycles, then on days 1,2,15 and 16 from cycle 13 to 18. *Results and Conclusions:* 18 pts with RRMM were treated with KRd at our institution from April 2016. 4 were excluded from analysis due to advanced disease (≥5 previous therapies). Of the 14 evaluated pts (median age 53, range 38-69; M/F=9/5), 8 received KRd after 1 previous therapy including bortezomib-based regimen followed by ASCT, 6 pts received KRd after 2 or 3 previous therapies. None was bortezomib refractory; 2 pts were previously treated with Rd; 1 was previously treated with anthracyclines. 8 were in paraprotein relapse while 6 in both paraprotein and clinical relapse. 2 pts were refractory to their last therapy. Lenalidomide was administered at 25 mg in 12 pts, at 15 mg in 2 pts. Median number of treatment courses was 4 (range 2-10). 5 pts experienced neutropenia (grade 3 in 4) resolved after G-CSF administration; febril neutropenia occurred rarely (3/14 pts). 4 pts had thrombocytopenia (grade 3 in 2) resolved after lenalidomide interruption or dose reduction, no major hemorrhage episode was documented. 3 pts showed grade 3 hypertension managed with appropriate anti-hypertensive therapy; 1 patient experienced grade 3 liver enzymes increase recovered after carfilzomib dose reduction. Overall response rate was 86% with CR in 3/14 pts (21%), VGPR in 4/14 (29%), PR in 5/14 (36%). After a median follow-up of 4 months (range 2-12) no severe cardiologic toxicity was observed; 2 pts died of disease progression, 12 pts were still on treatment.

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BORTEZOMIB-BASED THERAPY IN FRAIL MULTIPLE MYELOMA PATIENTS

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Background: Multiple Myeloma (MM) predominantly affects elderly patients (pts), more than 30% aged >75 yrs. They are more susceptible to side effects and often unable to tolerate full drug doses. Frail patients also are excluded from clinical trials and the impact of new drugs remains unknown. *Methods:* The aim of this single center retrospective study was to evaluate safety, efficacy and clinical outcome of front-line bortezomib based regimens in frail pts with newly diagnosed MM. Frail pts were defined using geriatric assessment (Palumbo A et al.) as older than 80 yrs or between 78 to 80 yrs with comorbidities. Pts were treated with 2 different bortezomib based regimens, for 6 cycles. VP: 5-weekly cycles containing sc bortezomib 1.3 mg/smq weekly and prednisone 25 mg every other day. VMP: 5-weekly cycles containing sc bortezomib 1.3 mg/smq weekly plus oral melphalan 9 mg/smq and oral prednison 40 mg/smq on days 1 to 4. MM treatment was selected on PS and comorbidities. *Results:* From August 2013 to December 2016, 39 consecutive MM pts were treated at our Institution Median age was 80 (range 78-89), M/F: 24/15. 12 pts (31%) had creatinine increased level (>1.5 x baseline), 12 (30%) had Hb < 10 gr/dl. 23 patients (60%) received VMP whereas 16 patients (40%) received VP. Early drug discontinuation (first 2 cycles) for toxicity was reported in 4/23 patients (17%) receiving VMP and in 3/16 pts (18%) receiving VP, mainly due to hematologic and neurologic side effects, grade 2-3. Treatment discontinuation after 2 cycles because of progression was reported in 1 (4%) pts in VMP and in 2 (12%) in VP regimens. After 6 cycles of treatment, 18 pts were evaluable in the VMP group and the ≥PR rate was 88% whereas in the VP group 11 pts were evaluable with ≥PR rate of 91%. The most common grade 3 AEs included infections (pneumonia), gastrointestinal toxicities and peripheral neuropathy. After a median follow-up of 13 months (range, 1-42), 12/39 pts (31%) died, mainly due to disease progression. In the VMP group, median PFS was 16 months (range 8-49 months), 11 pts relapsed and median time to next therapy was 18 months (range 11-34). Among those treated with VP median PFS was 7 months (range 3-19), 4 (36%) relapsed and only 2 started new treatment, median time to next therapy was 19 months. *Conclusions:* The best strategy for frail patients remains to be defined, nevertheless bortezomib based regimens with reduced dosage or in doublet are efficacy and safety in elderly.

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VALUE OF THE 18F-FDG PET-CT IN THE IDENTIFYING BONE INVOLVEMENT EITHER AT DIAGNOSIS OR DURING FOLLOW-UP OF PATIENTS AFFECTED BY MULTIPLE MYELOMA

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Background: Lytic lesions occur in the majority of patients with multiple myeloma (MM) and represent one of the criteria for starting therapy. In the past, whole-body X-ray (WBX) represented the method of choice for detecting skeleton abnormalities; today, magnetic resonance imaging (MRI), positron emission tomography (PET) and computed tomography (CT) have been adopted for their higher sensitivity. Our retrospective study was designed to compare PET-CT with other imaging techniques (WBX, vertebral column CT and MRI) at the diagnosis and during the follow-up. *Patients:* We enrolled 160 patients observed at the AOUP, Pisa, Italy, between January 1996 and December 2015. Eighty-three were male and 77 female; the median age was 70 years (range, 28-85), and half of them presented with low ISS risk score. Forty-five subjects were not eligible to high-dose therapy; 64% of them received bortezomib- and 23% melphalan-based regimens. Patients eligible to high-dose therapy received VAD, TAD or VTD and then one (88%) or two (12%) autologous transplants. *Results:* Overall, we compared 160 PET-CT, 233 WBX, 106 CT, and 85 MRI exams. At diagnosis, PET-CT allowed detecting skeletal involvement in 18% of cases negative by WBX, in 37% of those CT-negative, and in 10% of those MRI-negative. Sensitivity of PET-CT was superimposable to that of MRI (90%), and higher than that of WBX (60%) and CT (73%). Nevertheless, the specificity was lower for PET-CT and MRI (40%) in respect of CT (51%) and WBX (71%). Analogously to that observed at diagnosis, PET-CT during follow-up showed distinct advantages in terms of sensitivity compared to X-rays (83% *vs* 60%, respectively). In contrast, PET-CT sensitivity was comparable to that of CT and MRI. As at diagnosis, the specificity was higher for WBX (70%) than for CT, RM and PET-CT (40% for all of these). When PET-CT was correlated to the quality of response, it was significant only in the not transplanted cohort (>PR rate in PET-negative cases=67% *vs* 23% in the PET-positive group; p=0.016). Nevertheless, PET-CT positivity either at diagnosis or during follow-up did not impact on long-term OS and PFS. *Conclusions:* Our study showed that PET-CT and MRI would represent the techniques of choice in the assessment of bone involvement in MM patients in view of their high and comparable sensitivity. Moreover, PET-CT gives the possibility of a "whole body" analysis in exchange for higher "biologic" cost.

P204

SEVERE INFECTIONS IMPACT OVERALL SURVIVAL IN ACTIVE MULTIPLE MYELOMA PATIENTS

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Multiple myeloma (MM) is a hematological malignancy characterized by the proliferation of monoclonal plasma cells (PC) in the bone marrow. Both secondary immunodeficiency due to MM and specific therapy may account for the occurrence of severe infections (SI), defined by the need of hospitalization, that characterize the natural history of the disease. The aim of this study was to analyse the frequency and the major risks factors of SI in our cohort of patients affected by MM and to understand the impact of these events on MM patients' overall survival (OS). A cohort of 237 symptomatic MM patients followed for 14 years was retrospectively studied for the presence of SI over the time of the disease. Infections were classified as "not neutropenia related" (NNR) or "neutropenia related" (NR) according to the ANC> or <1,000/mm^3 respectively. Clinical characteristics as ISS and Durie-Salmon (DS) staging System, the percentage of bone marrow PC>60, age, hypercalcemia, renal failure, anemia and bone lytic lesions at diagnosis were considered in the study. In our cohort of patients, a total amount of 138 infectious events occurred in 91 active MM patients. Among these, 38 (28%) involving 34 patients were NR while the remnant 100 involving 68 patients NNR (72%); 11 patients experienced both NR and NNR. Major features presented at the time of diagnosis significantly associated to NNR SI were DS stage III (p=0.0002), ISS stage III (p<0.0001), bone marrow PC>60% (p=0.02), acute renal failure (p=0.0003) or MM presenting with at least three CRAB criteria (p=0.016). On the contrary, no significant risk factors were associated to NR SI mainly occurring after high dose chemotherapy and autologous stem cell transplantation (79%). Patients who experienced infectious events presented reduced OS as compared to other patients (p<0.0001) but those who developed exclusively NR SI showed better OS towards patients who experienced NNR SI (p=0.0011). Severe infections considerably impact natural history of MM patients and OS mostly in the setting of NNR SI. Therefore, immunoglobulin replacement therapy or antibiotic prophylaxis may possibly have a protective role in high risk patients characterized by ISS and DS stage III, bone marrow PC>60% and aggressive disease at the time of diagnosis.

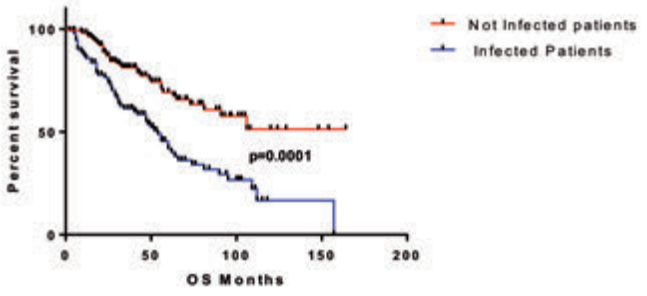

Figure 1.

P205

SAFETY AND EFFICACY OF NOVEL AGENTS IN VERY ELDERLY MULTIPLE MYELOMA PATIENTS: A REPORT BY THE RETE EMATOLOGICA PUGLIESE (REP)

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Multiple Myeloma (MM) is mainly a disease of the elderly. The aim of our study is to evaluate safety and efficacy of novel agents in patients with MM and 80 years and more. Patients from 9 Hematology Centers of the Rete Ematologica Pugliese "REP" were included in this study. Between January 2011 and December 2016, 75 patients (M/F: 44/31) with a median age of 82 years (range 80-91) were diagnosed as newly symptomatic MM. Of the entire study population, 40 (56%) patients showed an ECOG score <2. According to immunoglobulin heavy and light chain isotypes, patients had IgG-k(n=25), IgG- (n=16), IgA-k(n=16), IgA-(n=6), micromolecular k (n=8) and (n=4) chains. According to ISS, patients were classified as I (n=5) score, II (n=26) and III (n=44) score, respectively. When CRAB features were considered, bone lesions represented the most frequent (n=44, 58.7%) clinical manifestations. Majority of patients (n=52, 69.3%) showed at least 1 comorbidity requiring specific treatments. Patients were treated according to Bortezomib-based regimens (VMP, VCD and VD) (n=48; 64%), Lenalidomide-based regimen (RD) (n=8; 10.7%) and Thalidomide-based regimen (MPT) (n=6; 8%). Only 13 patients (18.3%) did not receive any novel agent. Based on IMWG criteria, 15 patients (20%) achieved a CR, 17 patients (22.7%) a VGPR and 17 patients (22.7%) a PR. 14 (18.7%) and 12 (16%) patients experienced a SD and a PD, respectively. As second line of treatment (44 patients), Bortezomib was used in 15 (34%) patients, Lenalidomide in 18 (41%) patients and Thalidomide in 3 (7%) patients. 8 patients (18%) were treated with old drugs (Melphalan, Cyclophosphamide or Bendamustine). Pomalidomide was used as third line-therapy in 3 patients. After 72 months (median 32.5 months) of follow-up, 36 (48%) patients remained alive with a median survival of 36 months and 26 (35%) died. Last follow-up from 13 patients was unavailable. Hematological and extra-hematological toxicities were similarly distributed and usually weak/moderate. Neuropathy was the most common toxicity reported (n=5, 6.6%). Of patients treated with only novel agents (n=62), hematological and extra-hematological toxicity was observed in 14% and 16% patients, respectively. We showed that all MM patients can be treated by novel agents independently of the age. Results from our study show that particularly very elderly and frail patients can benefit from these drugs by prolonging their life expectancy and maintaining a good quality of life.

P206

EFFICACY AND SAFETY OF LENALIDOMIDE, LIPOSOML DOXORUBICIN AND LOW DOSE DEXAMETHASONE (RDD) IN HEAVILY TREATED RELAPSED AND REFRACTORY MYELOMA PATIENTS: RESULTS OF LONG TERM FOLLOW-UP EXPERIENCE IN THE ERA OF NEWER DRUGS

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We conducted a phase II study, with long term follow-up,to assess the efficacy and safety of lenalidomide in combination with liposomal doxorubicin and low dose dexamethasone (RDd) in rrMM patients.Herein,we evaluated RDd as bridge regimen to transplant in refractory patients eligible for transplant. Between January 2009 and April 2016, 51 heavily treated patients were enrolled. Baseline patients are summarized on table1. RDd consisted of liposomal doxorubicin 30mg/m^2 on day 1,lenalidomide 25mg (days1-21) and 20mg dexamethasone(day1,8,15and22), of a 28 days cycle.Six cycles were performed.The ORR was 78% (56%VGPR and 22%PR), table2.Median TTR was 3,7 months (range0,47-11,9). According to univariate analysis, ISSstage>II (p=0,09) and prior treatment with thalidomide (p<0,06) seemed to reduce ORR.Prior bortezomib treatment positively affected ORR (50%*vs*.79%; p<0,05).Prior ASCT (p=0,72) and older age (p=0,20) not influenced ORR. At 34 months of follow-up, median TTP was 21,6 months(95%CI:14 28) and 62% at 1,5 years. Median PFS was 19 moths (95%CI:14-23); the 1,5yPFS was 57%.In univariate analyses,response<VGRP post 6RDd worsened TTP (14*vs*.32 months,p=0.001). Prior ASCT (16*vs*.24 months,p=0,07), prior bortezomib (20*vs*.15 months, p=1,70) or thalidomide (17*vs*.27 months,p=0,94), older age (42*vs*.18 months, p=0,12), >=2 prior therapies (35 months *vs*.76,p=0,07) not influenced TTP. The median OS was 45 months(95%CI:29-58) and 1,5yOS was82%.Responding patients,not eligible to transplant, received additional three RDd cycles as consolidation and ORR was96%(16%CR, 60%VGPR,20%RP).During manteinance with 10mg Lenalidomide, the ORR improved to 100% (47%CR,37%VGPR, 16%RP). In refractory patients eligible to transplant,63% RDd chemosensitive patients allowed auto transplant with 1,5y PFS of 60%. The main 2-3 grade side effects were anemia(34%),thrombocytopenia

(33%), neutropenia(69%), and infection (24%).In conclusion,the RDd was manageable and cost-effective,marking high ORR and a continual clinical benefit during consolidation and maintenance treatment, as in elderly than in heavily pretreated patients.Notably in refractory patients,RDd was effective as bridge regimen to transplant. With next availability of drugs with novel mechanism of action,RDd could seem overcome but its cost-effective results are very timely and might represent a further opportunity in the therapeutic landscape of difficult-to-treat patients.

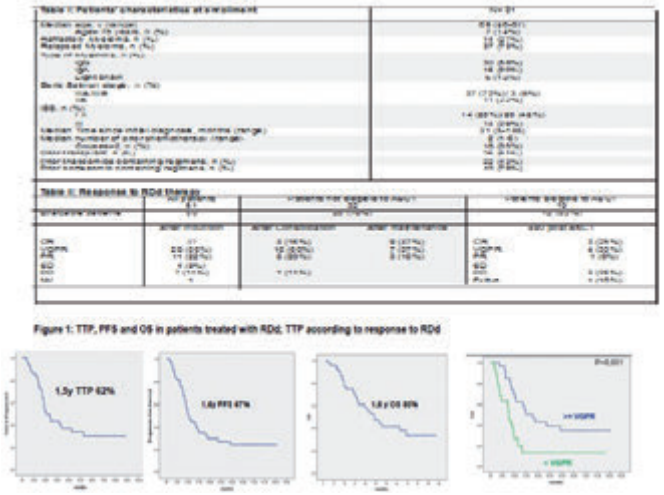

Figure 1.

P207

CD200 EXPRESSION AS SPECIFIC MARKER OF ABNORMAL PLASMA CELLS IN MYELOMA PATIENTS

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Treatment of multiple myeloma has changed markedly in the past decade due to the development of new drugs such as proteasome inhibitors and Immunomodulants and recently with monoclonal antibodies, which have greatly improved the outcome of disease. CD200, which was initially described as the OX-2 tumor antigen, is a transmembrane glycoprotein that is a potential therapeutic target due to its role in immune regulation and tolerance, but its role in Multiple Myeloma remains questionable. The aim of this study was to determine the diagnostic significance of CD200 expression in different phases of clinical active Myeloma. We retrospectively reviewed the records of 101 patients diagnosed with MM at Pisa and Siena University between 2012 and 2017. We analyzed the CD200 expression on pathological (Plasma cells) PCs, from bone marrow samples of patients. The clinical and pathological features of the CD200-positive and CD200-negative groups were similar. Immunophenotyping was carried out by a 6-color method, using a FacsCanto II cytometer and the FacsDiva software. PCs were identified as CD138+/CD38+ events after an initial gate which included events with low SSC in the CD45/SSC cytogram. The MoAb panel also included CD19, CD20, CD117, CD56, cytoplasmic light chains K and Lambda. PerCP-Cy5.5-conjugated CD200 was evaluated on phenotypically abnormal PCs (*i.e.* CD19-, CD45- or dim), which were resulted to be clonally restricted.The positivity cut-off was arbitrarily fixed at 20%. CD200 was expressed on PCs in 61% of all cases (62 of 101 pts, see table I), similarly to previous findings in patients evaluated at diagnosis, but when we selected specific subclasses of patients with different status of disease, we confirmed that CD200 expression remained quite expressed and stable: in particular we detected 70% of CD200+ in CR pts, 71% in VGPR, 63% in PR, and 58% in refractory/relapsed pts. CD200 expression was not associated with survival in our cohort but we also showed a trend to reduction of CD200 expression in partial responders and refractory/relapse patients, according to previous data where the loss of CD200 expression in PCs had been associated with a more clinically aggressive disease. CD200 expression in PCs can be not only a diagnostic marker but it can be more useful in follow up analysis due to its stability and could represent a potential marker of worse prognosis.

Table 1.

Table I Pts Characteristics	Total	CD200+
	101	62/101
Sex		
	Male	58(57%)
	Female	43(43%)
Clinical status		
	MM at diagnosis	42/101
	MM after treatment	59/101
	in VGPR/CR/PR 72%	
	in SD/PD 28%	

P208

SMOLDERING MULTIPLE MYELOMA : THE ROLE OF RISK MODELS IN IDENTIFYING HIGH RISK PATIENTS IN REAL LIFE

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Smoldering Multiple Myeloma (SMM) is characterized by a clonal bone marrow plasmacells (BMPC) proliferation ≥10% and/or a serum monoclonal component ≥ 3 g/dl and absence of CRAB symptoms. Although it is a pre-neoplastic state of MM, the standard of care today is not to treat. Thus, it is of great relevance to have tools which allow to identify those patients (pts) with a high probability of progression to precociously start treatment in order to prolong time to progression (TTP) and to avoid ominous disease related complications. Several risk models for SMM have been identified, especially from Mayo Clinic. Another tool comes from consensus published in 2014 identifying the so called SLIM CRAB(BMPC ≥60% or serum free light chains [FLC] ratio ≥100 or at least one lesion at MRI) that characterize ultra high risk SMM, with progression of 80% at 2 years. Aim of our study is to apply these risk models in a cohort of 75 SMM pts diagnosed from 2000 to 2015. We firstly evaluated the effect of variables at diagnosis on progression-free survival by Cox proportional hazard model. As reported in Table 1, among all variables, only BMPC percentage at diagnosis is significant for progression(p=0.017).

Table 1.

Sex	F 34/75 (45.3%) M 41/75 (54.7%)
Median age	63.1 yrs (34.5-84.2)
CM type	IgA 14/74 (18.9%) IgG 56/74 (75.7%) biclonal 1/74 (1.4%) light chain 3/74 (4 %)
First Mayo Risk model	Risk group 1 (MC ≥3 g/dl and BMPC≥10%) : 9/71 (12.7%) Risk group 2 (MC < 3 g/dl and BMPC≥10%) : 62/71 (87.3%) Risk group 3 (MC ≥3 g/dl and BMPC < 10%): 0/71(0%)
Second Mayo Risk model • MC ≥3 g/dl • BMPC≥10% • Serum FLC ratio between <0.125 or > 8	Risk group 1 (1 risk factor) : 24/50 (48%) Risk group 2: (2 risk factors) 23/50 (46%) Risk group 3: (3 risk factors) 3/50 (6%)
Hemoglobin	13.4 g/dl (10.1.-17.1)
creatinine	0.87 mg /dl (0.54-2.9)
calcium	9.4 mg/dl (8.7- 12)
LDH	256 mU/ml (139- 1014)
monoclonal component	2,2 g/dl (0.47- 5.5)
albumine	4.28 g/dl (3.18- 5.4)
Urine immunofixation	positive 27/59 (45.8%) negative 32/59 (54.2%)
Urine immunofixation	kappa: 14/26 (53.8%) lambda: 12/26 (46.2%)
Urine 24 HR protein	0.116 g/24 HR (0-0.62)
c- terminal telopeptide	0.414 ng/ml (0.051-0.967)
FLC kappa/lambda ratio	7.1 (1.05-3093)
B2microglobuline	2331 mcg/L (1320- 13000)
Bone marrow plasma cells (%)	15 (10-55)
Skeletal survey at diagnosis	45 X- ray : no lysis 21 CT scan: no lysis 12 MRI: no lysis

With a median follow up of 4.6 years (0.1- 15), 17 of 75 pts (22.7%) show a progression with a CRAB and median TTP is 3.6 years (0.25-15.2).The cumulative risk of progression is 4.2%(95%CI: 1.1%-10.6%) at 1 year from diagnosis; 9.1% (95%CI: 3.7%-17.5%) at 3 years and 22.7% (95%CI: 12.3%-35.1%) at 5 years. With the first Mayo Clinic risk model of 2007, 3/9 have a progression in group 1 with a median TTP of 19.2 months and 13/62 in group 2 with a median TTP of 46.9 months (p=0.313), no pts in group 3. Applying the second Mayo Clinic risk model of 2008, 4/24 have a progression in group 1 with a median TTP of 45.1 months; 4/23 in group 2 with a median TTP of 29.4 months; 2/3 had a progression in group 3 with a median TTP of 12.4 months (p=0.305). 5/28 pts (17.8%) are ultra- high risk SMM, all for abnormal FLC ratio; 2/5 progress with a median TTP of 12.4 months (5.6–19.2). Being in this group of pts is, in multivariate analysis, a risk of progression itself (p=0.008). In conclusion, risk models are useful in identifying pts with a higher risk of progression in whom a different monitoring is indicated. In particular, ultra-high risk SMM and the second Mayo risk model seem to be more reliable. In multivariate analysis, BMPC percentage is the main risk factor for progression.

P209

STEM CELL MOBILIZATION EFFICIENCY OF HIGH DOSE CYCLOPHOSPHAMIDE COMPARED TO INTERMEDIATE DOSE CYCLOPHOSPHAMIDE IN MULTIPLE MYELOMA PATIENTS

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Mobilization with cyclophosphamide (CTX) plus G-CSF is the standard of care for newly diagnosed multiple myeloma (MM) patients planning to undergo autologous stem cell transplantation (ASCT). Recently, high dose CTX (4-7g/mq) has been substituted by intermediate dose (2-3g/mq) with the aim of reducing toxicity and allowing the management of the entire procedure in outpatient clinic. The aim of our study was to compare the mobilization efficiency in 47 MM patients receiving high-dose CTX (4 g/mq) plus 5 mcg/Kg G-CSF with 57 patients treated with intermediate dose cyclophosphamide (2-3 g/mq) followed by 5mcg/Kg G-CSF. There were no significant differences in MM features, inducton therapy and response between the two cohorts, but median age of the CTX<4 group was significanly older than CTX4 group (62 *versus* 58 years, p=0.01) Mean CD34+ cells count in PB before collection was higher in the CTX4 cohort compared with CTX<4 (115/L *vs* 79/ L, p=0.08) while mean leukocyte count in PB was higher in the CTX<4 group ($23x10^3$/L *vs* $19.5x10^3$/L, p=0,07). Plerixafor "on demand" was required in 2/47 patients (4%) of the CTX4 cohort and in 8/57 patients (14%) of the CTX<4 cohort (p=0.09). Only 1 patient in the CTX<4 cohort failed mobilization after Plerixafor. Median CD34+ cells harvest was $9x10^6$/Kg (range 2.4-56.2) in the CTX4 group and $8x10^6$/Kg (3.5-21.8) in the CTX<4 group (p=0.09). However, CD34+ cells mean vitality evaluated by Trypan Blue techinique was significantly lower in CTX<4 group compared to CTX4 group both immediately after collection (73% *vs* 95%, p=0,02) and at the time of stem cell reinfusion (71% *vs* 93%, p=0.01). However, all patients underwent ASCT and achieved a successfull engrafment. Toxicity was lower in the CTX<4 group compared with to CTX4 group (hemorragic cystitis: 21% *vs* 10%; major infections needing hospitalization: 5% *vs* 4%; grade III-IV WHO neutropenia: 96% *vs* 78%; grade III-IV WHO piastrinopenia and 48% *vs* 35%). In summary, we showed that intermediate dose CTX was associated with a higher proportion of poor-mobilizers, but the administration of plerixafor 'on demand' achieved an adequate CD34+ cells collection in all but 1 patient. Even if vitality of CD34+ cells harvest was lower in the CTX<4 group, all patients could proceed to ASCT, with a successful engrafment.

P210

DYNAMIC IMMUNOHISTOCHEMICAL EVALUATION OF MARROW MICROENVIRONMENT MODIFICATIONS IN PATIENTS WITH SMOLDERING MYELOMA

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Aim: Our aim was to identify a progressive dysregulation of marrow microenvironment in sequential samples of SMM patients. Secondly, we hypothesized a difference in the microenvironment of the patients with progressed SMM *versus* those with stable SMM. *Methods:* We performed immunohistochemical analysis of bone marrow samples of 16 patients affected by SMM at time 0 (16 samples) and at +24 months (+/- 4 months, 16 samples). Half of these patients developed MM at 24 months (progressed SMM), the other half remained asymptomatic (stable SMM). Immunohistochemical panel comprised the following markers: microenvironment cell (CD138, CD4, CD8, CD3, CD45, CD56, CD68), loss of immunogenicity (PDL1, PDL2, PD1, LAG3, CTLA-4, IDO), loss of antigenicity (HLA-DR). Immunogenicity and antigenicity markers expression was described as percentage on the total of marrow plasma cells and non-plasma cells separately. A first analysis compared the samples of the whole cohort at time 0 and +24 months (32 samples, paired t-test). A linear correlation was performed to study the expression of T cell inhibitory ligands on plasmacells and their respective receptors on marrow non-plasma cells (HLA-DR with LAG3, PDL1 with PD-1). A second analysis compared, at time 0 and +24 months, the samples of stable SMM *versus* progressed SMM (16 samples each analysis, t-test). *Results:* In the first analysis, we found a significant increase between time 0 and +24-month samples of CD4+ (11% *vs* 17%, p<0.01), CD8+ (15% *vs* 18%, p<0.01), CD4/CD8 ratio (0.75 *vs* 0.94, p<0.01), PDL1 on plasmacells (1% *vs* 12%, p=0.03), HLA-DR on plasmacells (7% *vs* 10%, p=0.04), HLA-DR on non-plasmacells (29% *vs* 39%, p=0.01), LAG3 on non-plasmacells (4% *vs* 10%, p=0.04, figure 1). Interestingly, we found a significant correlation between LAG3 and its ligand HLA-DR (r=0.47, p=<0.01). In the second analysis we compared stable SMM *versus* progressed SMM at time 0 and at +24 months. At time 0, we found only an increased CD68-KP1 expression in favor of stable SMM group (28% *vs* 23%, p=0.01). At time +24 months, no differences were observed but an increased plasma cell marrow infiltration in the progressed SMM group (50% *vs* 26%, p<0.01). *Conclusions:* We observed an increase in inflamed microenvironment markers (increase in CD4+ and CD8+ cell count in favor of CD4+ population and HLA-DR expression) during the course of SMM. Expression of T cell inhibition markers (PDL1, LAG3) was significantly augmented during disease progression.

P211

BORTEZOMIB-BASED REGIMENS AS FIRST-LINE OR SALVAGE THERAPY FOR POEMS PATIENTS

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POEMS is a paraneoplastic syndrome due to a plasma cell (PC) dyscrasia. Diagnostic criteria have been proposed by the IMWG. There are no established treatments and no randomized controlled clinical trials of treatment exist in the available literature. sVEGF is a reliable marker of the disease activity. We report on 4 patients with POEMS syndrome (according to the IMWG criteria), treated with Bortezomib-based regimens; three of them previously relapsed after Lenalidomide-Dexamethasone (RD). Patient #1, 33, M. At the time of the diagnosis, sVEGF was 2.822 ng/L (n.v. 62-707). The patient underwent several lines of therapy including RD but he eventually relapsed; sVEGF was 9.860 ng/L. After 6 cycles of the CyBorD regimen (Cyclophosphamide 300mg/m^2, Bortezomib 1.5mg/m^2, Dexamethasone 20 mg on days 1, 8, 15 and 22 of a 28-day cycle), sVEGF dropped to 586ng/L; clinical signs and symptoms slowly improved. The patient was given CyBorD chemotherapy every three month as a "maintenance" strategy. After 34 months, neurologic condition improved, sVEGF level is in the normal range. No toxicity was observed. Patient #2, 46, M. At the time of the diagnosis, sVEGF level was 2.000 ng/L. The patient underwent two lines of therapy including RD, achieving a partial response and normalization of sVEGF levels. Upon relapse, the patient was started on Bortezomib-Dexamethasone regimen (Bortezomib 1.5mg/m^2, Dexamethasone 20 mg on days 1, 8, 15 and 22 of a 28 day cycle). After 6 cycles, the patient was given Bortezomib at 1.5mg/m^2 every 14 days. After 25 months the

patient achieved a little neurological improvement; sVEGF levels are in normal range. No significant toxicity was observed. Patient #3, 47, F. At the time of the diagnosis, sVEGF level was 10.640 ng/L. She underwent two lines of treatment achieving a partial response. Upon relapse, sVEGF levels was 29.558 ng/L. The patient was started on CyBorD regimen with partial clinical improvement; sVEGF dropped to 6.840 ng/L. The patient is currently undergoing an ASCT following high-dose melphalan. Patient #4, 52, F. At the time of the diagnosis, sVEGF levels was 15.176ng/L. She was started on Bortezomib-Dexamethasone regimen. After 3 cycles, sVEGF levels dropped to 3.269ng/L, with a significant clinical and instrumental (electromyography) response. In our patients, Bortezomib-based regimens proved to be safe and effective both as first-line and as salvage therapy. No significant neurological toxicity was observed.

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CARFILZOMIB-LENALIDOMIDE-DEXAMETHASONE IN THE MANAGEMENT OF LENALIDOMIDE-REFRACTORY MULTIPLE MYELOMA: A REAL-LIFE EXPERIENCE

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Carfilzomib is an epoxyketone proteasome inhibitor of second generation, proved to be effective in relapsed and refractory Multiple Myeloma (rrMM). In this retrospective observational trial, it has been evaluated efficacy and tolerance of Carfilzomib, in combination with lenalidomide-dexamethasone (KRD) as salvage regimen in patients with rrMM, refractory to lenalidomide,whose prognosis is particularly severe. 21 patients (12 M/9 F), with rrMM,median age at diagnosis 62 years (r. 47-75), median age at start of treatment 65 years (r. 53-81) treated with several lines of treatments (median 3, r. 2-10), underwent to KRD regimen (as ASPIRE trial schedule) for a median treatment cycles of 2 (r 1-8). ISS was equally distributed, and cytogenetic was evaluable in 8 patients, and in particular one del13q14 1qgain, one del 13q14 and one t(11;14). 86% of patients had previously been treated with bortezomib and IMIDs. 57% of them had undergone at least to a single autologous SCT. Carfilzomibwas well tolerated, with grade 2 anemia in 28% of patients,managed by ESAs, without necessity of blood transfusions; 9.5% grade 3 neutropenia (pegfilgrastim in primary prophylaxis was given, no ospedalization was required, no septic shocks were observed); 33% grade 2, 19% grade 3 and 5% grade 4 thrombocytopenia, without hemorrhagic events and necessity of transfusions. Concerning severe extra-hematologic toxicity, it was observed grade 1 pneumonia in 47% of patients, treated by common antibiotic drugs; grade 2 hypertension in 24% of patients; grade 3 arrhythmias in 5% of patients; grade 2 dyspnea in 5% of patients; grade 1 fatigue in 9.5% of patients. All patients were carefully monitored by expert cardiologists. According to IMWG criteria, after a median follow-up of 3 months (r.1-13), ORR was 66,7%(14/21: 8 VGPR, 6 PR) with 3progressive diseases and 2 patients in stable disease, which can be considered as an impressive result in this subset of rrMM patients, refractory to lenalidomide. In particular, for 1 patient, KRD was, after having achieved at least a PR, a bridge to second autologous SCT. Median time to response was 2 months (r.1-4), median OS from diagnosis was 47 months (r. 9-170), median OS from start of Carfilzomib was 3 months (r. 1-13). KRD has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to all available therapeutic resources, also lenalidomide, and, in particular cases, it could be considered as a bridge to a second autologous or allogenic SCT.

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SINGLE SHOT MEDIUM MELPHALAN IN RELAPSED MM PATIENTS: A RETROSPECTIVE, SINGLE CENTER EXPERIENCE

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Multiple myeloma (MM) patients refractory to proteasome inhibitors, IMIDs or both, have an extremely poor prognosis. They frequently fail to respond to further therapies and represent a major challenge in everyday clinical practice. With this in mind, we treated 12 patient with relapsed MM with a single shot of medium dose melphalan (60 mg/m2) between October 2010 and January 2016. The median age was 72 years (range 62–79) and the median time from initial diagnosis until melphalan treatment was 51 months (range 24–144). Patients were heavily pretreated with a median number of 3 prior lines of therapy. All patients were refractory to the previous therapeutic regimens and had failed to respond or were refractory to regimens containing bortezomib. Seven patients (84%) had previously received at least one IMiD, 8 (67%) autologous stem cell transplantation and 1 allogeneic stem cell transplantation. The patients included in the series were not eligible for any clinical trial available at the Institution. All patients gave informed consent. All patients had cytopenia (anemia, neutropenia and thrombocytopenia). We observed 3 cases of gastrointestinal toxicity (1 bleeding, 1 subocclusion, 1 mucositis grade IV sec. WHO), 3 cases of clinically documented infection (1 Escherichia coli bacteremia, 1 fever of unknown origin, 1 erysipela) and 2 deep vein thrombosis. Response was assessed between six and eight weeks after melphalan therapy. Overall, 10 out of 12 patients had a response (1 complete response, 3 very good partial response, 2 partial response and 4 stable disease); only 2 had progressive disease. Median overall survival was 11 months (range 2 -37). 10 of 12 patients relapsed after a median time of 5 months (range 2-12). Concerning two patients not relapsed, 1 patient died in partial response 8 months after therapy of other causes; 1 patient is still alive, in complete remission 20 months after melphalan. He underwent ASCT and maintenance with lenalidomide. Many patients refractory to proteasome inhibitors and IMiDs are probably still sensitive to alkylating agents and could be rescued with medium dose melphalan. Even in this era in which several novel drugs became available, single shot medium dose melphalan could be an affordable and safe therapy, able to control aggressive relapse, and to reduce disease burden prior to targeted therapy.

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ROLE OF SERUM FREE LIGHT CHAIN VS BENCE JONES MEASUREMENT IN LIGHT CHAIN MULTIPLE MYELOMA (LCMM) AT DIAGNOSIS, DURING TREATMENT AND FOLLOW-UP FOR RESPONSE EVALUATION AND RELAPSE DETECTION

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According to IMWG recommendations for response assessment in multiple myeloma (MM), serum free light chain (FLC) measurement should only be used 1) in light chain multiple myeloma (LCMM) when Bence Jones protein (BJP) is not measurable (<200 mg/24h) and 2) to define a stringent complete response (sCR). However, data are available suggesting that FLC could be a more sensitive tool than BJP for minimal residual disease assessment and an earlier indicator of progressive disease (PD). Aim of our study was to retrospectively compare FLC and BJP results in LCMM pts at diagnosis, during treatment and follow-up. Serum and urine samples were collected from pts affected with plasma cell dyscrasia referred to our institution between Feb 2012 and Dec 2013. Protein electrophoresis was performed using Capillarys II, immunofixation using Hydrasys II, FLC were measured on Immage 800 nephelometer using Freelite reagents. We analized samples from 387 pts with positive serum and/or positive urinary immunofixation and/or

abnormal FLC ratio. Among them, 43 symptomatic LCMM pts were identified having both FLC and BJP measurement at baseline (MM diagnosis or first relapse). Lab results were evaluated at baseline, monthly during therapy and every 3 months during follow-up. Median duration of monitoring for the whole group was 42 months. ASCT was performed in 30% of pts previously treated with PIs (81%) and/or IMIDs (40%) or chemotherapy (9%). FLC or BJP were not available in 10% of 872 pair of samples from 43 pts. In 10% of cases FLC ratio was abnormal with increased involved FLC without any detectable BJP (FLCr+;iFLC+;BJP-); the opposite (FLCr-;iFLC-;BJP+) occurred in 1%. BJP was not measurable at baseline in 6/43 LCMM pts who were thus excluded from further analysis. Median time to BOR was 3 months by both FLC and BJP. Among 37 pts evaluable for best overall response, 6/37 had CR according to BJP but not FLC and 5/6 progressed after 2-8 months. 21 pts progressed during follow-up: PD was detected only by FLC in 4, only by BJP in 1. Both tests were able to detect PD in 16 pts. In our series only 1 case showed BJP-PD according to IMWG occurring earlier than FLC-PD but was considered not clinically significant. On the contrary 5 pts in BJP-CR clinically progressed within few months without having reached FLC-CR. In conclusion, FLC assessment appears to be more sensitive in MRD and early relapse identification.

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REAL-WORLD EXPERIENCE OF LENALIDOMIDE/DEXAMETHASONE TREATMENT FOR REFRACTORY/RELAPSED MULTIPLE MYELOMA (RRMM) PATIENTS

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Background: the therapeutic scenario of patients with multiple myeloma has changed dramatically over the past ten years. Among the protocols registered for the treatment of RRMM, Lenalidomide/dexamethasone (Rd) is a widely used therapeutic option. *Aims:* Our retrospective analysis is a study that will provide long-term follow-up data on progression survival (PFS), and safety of Rd for a period of treatment of almost ten years. *Methods:* From October 2007 until March 2017, 115 RRMM patients have been included: 65 males, 50 females; median age was 70 years (range, 41-89); 62 IgG, 31 IgA, 1 IgD, 18 light chains, 2 non secretory. The median time from diagnosis was 32 months (range 2-238), 17 patients (15%) had renal failure and 41 patients (36%) had received autologous bone marrow transplantation (ABMT) as first or subsequent rescue lines. In 61 patients we used Rd as second line (53%), in 37 as third line (32%), in 17 beyond the third line (15%). Some different therapeutic combinations have been undertaken, including 2 CPR (Cyclophosphamide+pred+R), 1 RAD (Adryamicin+Rd), 1 MPR (Melphalan+pred+R) and 5 KRD (Carfilzomib+Rd). The average number of cycles was 8 (range, 1-76) and 19 patients were treated with 24 cycles. 84 patients (73%) discontinued therapy: 80 due to desease progression, only 4 patients (5%) due to adverse events (AEs). 31 patients (27%) are still in active treatment. *Results:* according to EBMT criteria, the ORR (≥PR) was observed in 80 patients (70%); 7 (6.5%) achieved a CR, 30 (26%) a VGPR, 43 patients (37.5%) had PR, 30 patients (26%) SD, while 5 patients (4%) progressed in the course of therapy. AEs occurred in 88% of patients, mainly related to myelosuppression. The incidence of second primary malignancies was 4%, peripheral neuropathy was observed in 14% of patients, DVT in 1%. Extramedullary relapse occurred in 1% of patients and permanent discontinuation for toxicity occurred in 4% of the study population. We demonstrate, the importance of obtaining at least a PR response, with respect to a SD ($p<0.0001$); age and IR at diagnosis did not affect the quality and duration of response, as well as ABMT as previous therapy. Positive impact on PFS were observed for male gender (p. 0.02), ISS, fewer lines prior to Rd (p. 0.009), and fewer AEs, rather than a reduction of dosage. *Conclusions:* The results of our study support the data from Phase III previous studies, showing that Rd is effective and generally well tolerated in patients with RRMM.

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AUTOLOGOUS STEM CELL TRANSPLANTATION IN ELDERLY MULTIPLE MYELOMA PATIENTS

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Background: Autologous stem cell transplantation (ASCT) is currently approved as "gold standard" first line treatment for multiple myeloma (MM) patients (pts) under 65 year old, furthermore it is considered feasible in fit elderly pts in retrospective studies. *Methods:* To evaluate the tolerability and the efficacy of high dose chemotherapy followed by ASCT we retrospectively analyzed consecutive MM pts aged 65 or older who underwent upfront ASCT at our institution from January 2009 to December 2016. *Results:* We analyzed 35 pts; 21M/14F, median age 66 (range 65-70). Induction therapy was bortezomib in combination with dexamethasone, VD, in 7, or VD plus thalidomide in 26 pts, for a median of 4 cycles (range 3-6), 2 pts received thalidomide plus dexamethasone (6-12 cycles). Peripheral blood stem cells (PBSC) were collected after high-dose cyclophosphamide (2g/sqm in 2 pts, 3g/sqm in 11 pts, 4 g/sqm in 22 pts) plus G-CSF; plerixafor was administered in 4 pts. 3 pts received lenalidomide and dexamethasone to improve the depth of response before ASCT. At the time of conditioning, among 34 evaluable pts, 8/34 pts were in complete response/stringent complete response (CR/sCR), 19/34 in very good partial response (VGPR), 5/34 in partial response (PR) and 2/34 in stable disease (SD). The conditioning regimen consisted of melphalan 140 mg/sqm in 11 pts or 200 mg/sqm in 24 pts. A median number of $4.11x10^6$ CD34+ cells/Kg was reinfused (range 2.09-10.44). The most frequent complication was fever (9 pts) with gram negative bacteremia documented in 3/9 and gram positive bacteremia in 1/9. Other complications were represented by 1 case of atrial fibrillation and 3 cases of pneumonia and 1 case of VZV reactivation. All 35 pts achieved neutrophils recovery after a median of 12 days (range 8-25) and platelets recovery after a median of 13 days (range 8-45) after transplant. No grade 3-4 toxicities were recorded. No transplant-related mortality was recorded within 100 days post transplantation. Three months after ASCT, among 28 evaluable pts, 10/28 pts were in CR, 14/28 pts in VGPR and 4/28 pts in PR. Three pts underwent tandem ASCT. After a median follow-up of 32 months (range 5-99) among 33 evaluable pts, 20 experienced disease relapse and 7 deaths occurred. Median PFS and OS were 21 and 40 months. *Conclusions:* ASCT is an effective and safe first-line treatment approach also in elderly MM. A careful pts selection reduce the toxicity of the procedure.

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USE OF VCD THERAPY IN ELDERLY PATIENTS (FIT AND UNFIT) WITH NEWLY DIAGNOSED MULTIPLE MYELOMA. RESULTS OF A SINGLE CENTER

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Plasma cell myeloma is a clonal disorder of malignant plasma cells and is a disease of the elderly, with a median age of onset of 70 years. Elderly patients comprise a heterogeneous group of variable fitness from the very frail to the remarkably fit. Adequate assessment of fitness prior to treatment is important: inadequate assessment will lead to instances where frail patients are overtreated, and fitter patients are undertreated. From January 2013 to October 2016 we detect 27 elderly patients with symptomatic MM. After geriatric assessment (ADL, IADL, CCI, PS) 5 were classified as Frail and received a less aggressive therapy,Of the remaining 22 patients, 17 (77,3%) were classified Fit and and 5 (22,7%) Unfit and received the VCd cycle: bortezomib 1.3 mg/m^2 intravenously or sc and cyclophosphamide 300 mg/m^2 intravevously on days 1, 8, 15 and 22 and dexamethasone 20 mg orally on days 1-2, 8-9, 15-16 and 21-22 on a 36 day cycle for nine cycles. At the end of the fourth cycle a disease revaluation was made. In case of no response or progression this therapy was stopped. All patients were treated also with Bisphosphonate. The mean age of the patients was 76,3+4,3years, 2 (9,1%), 8(36,3%) and 12 (54,5%) were ISS I, II, and III respectively. The response was assessed according to the IMWG criteria (Duriel, 2006). After 4 cycles 4 patients (18,2%) stopped therapy: 2 were non responder and 2 had a progression of disease;18 patients had at least a partial response and were treated with 9 cycles of VCd. For these 18 patients the ORR at the end of therapy was 82%, with 72% of RC or sRC and 28% of RP. At 24 months the OS was 57,1% and the PFS was

38,2%. In 7 patients there has been an infective complication, but only in one (peritonitis due to diverticulars drilling) hospitalization was necessary. Three patients who didn't have prophylactic therapy with Acyclovir had herpes zoster infection. No patient had hematologic side effects of grade 3 or 4. Three patients (13,6%) had a grade 2 neuropathy, and one had osteonecrosis of the jaw.Our results are similar to those obtained by Reeder on a more younger population (Leukemia 2009), while are a little bit better than those reported by Mateos MV (Lancet Oncol 2010) with VMP and better than those published by Tuchman G (J Geriatr Oncol. 2017) on an elderly population. We believe that this three-drug regimen can be considered a valid therapeutic opportunity with manageable toxicity in an elderly population fit and unfit.

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PRECENCE OF BONE LESIONS AND ALKALINE PHOSPATASE (ALP) IN MULTIPLE MYELOMA (MM) AND SOLID TUMORS

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Background: alkaline phosphatase (ALP) is an enzyme localized in different tissues and its levels may increase in diseases with skeletal involvement. *Aims:* To compare ALP levels in patients with Multiple Myeloma and osteolytic lesions, and in patients with solid tumors (breast, prostate, lung, stomach, kidney and colon) and osteolytic, osteoblastic and mixed (osteolytic and osteoblastic) bone metastatic lesions. Patients and *Methods:* From 1991 we collected 400 patients with MM and 308 patients with solid cancer with bone involvement. We grouped patients according to the metastases type (osteolytic, osteoblastic mixed), number of metastasis (1, 2-3, more than 3), ISS and D&S Stage. Among patients with solid tumors 45% had lytic lesions, while 31,5% had osteoblastic lesions and 23,5% had mixed lesions. Comparing MM *vs* bone metastasis from solid cancer respectively 64.5% *vs* 59% had normal ALP values, while 29% *vs* 28% had <2xUNL, and 6.5% *vs* 13% had >2UNL values of ALP. Patients with MM had significantly lower ALP values when compared to patients with osteoblastic bone lesions (P<0.05). In particular, comparing ALP levels of MM patients with the levels observed in cancer patients with >3 osteoblastic lesions difference is even more significant (P<0.01). Moreover, patients with Breast and Prostate cancer had ALP values significantly higher than MM pts (P<0.05 and P<0.05 respectively). In the group of patients with solid tumors, ALP levels were significantly higher in those with >3 osteoblastic lesions than in those with only osteolytic lesions (P<0.05). *Conclusions:* These preliminary results indicate that ALP should be part of the initial work up in pts with bone lesions. This simple and cheap test, if normal or reduced in presence of osteolytic bone lesions, suggests an initial complete protein study including serum and urine protein electrophoresis associated to Bone Marrow aspirate, in case of the presence of a paraprotein.

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EVALUATION OF FREE LIGHT CHAINS (FLC) IN SAMPLES OF SERUM AND BONE MARROW OF PATIENTS WITH MULTIPLE MYELOMA

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Background: Serum and urinary FLCs are evaluated in patients with Multiple Myeloma (MM) at diagnosis, to monitor response during therapy and follow-up. In a previous case study, we evaluated Bone Marrow FLC concentration and ratio in 25 patients (pts) with Multiple Myeloma Multiple, comparing the results with serum levels. No differences were observed. Only in one patient bone marrow K/L ratio was markedly higher than serum value. The patient obtained a nCR and also the bone marrow K/L ratio became normal. *Patients and Methods:* We have extended this study to 48 patients: 29 with active disease and 19 responders (>VGPR). FLC dosage was performed with Binding Site method (K/L normal range 0,23 - 1,65). *Results:* In all patients studied bone marrow and serum values were not significantly different. In 30 patients, bone marrow and serum K/L ratio ranges between 0.66 and 1.33. In 9 patients this ratio is less than 0.66 and in 8 it is higher than 1.34. However, no patient had normal K /L ratio in a type of sample and altered in the other. We did not find any differences between two types of samples and in the results obtained in K and L Myeloma. *Conclusions:* These data suggest that FLC evaluation in bone marrow does not add any useful information to serum evaluations, therefore this procedure can be considered superfluous, except, maybe, in those cases where the serum K/L ratio at diagnosis is normal.

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DIFFERENCES IN LABORATORY TESTS: SERUM FREE LIGHT CHAINS AND IMMUNOPARESIS IN PATIENTS WITH IGG MGUS AND IGA MGUS

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Background: IgA monoclonal gammopathy of undetermined significance (MGUS) are associated with a higher risk of progression to Multiple Myeloma. We indirectly confirm this data, in fact in our cohort of patients (pts), IgA MGUS are 13% among total MGUS and they are 20% among total Multiple Myeloma. Aim of study: To verify if patients with IgA MGUS have different laboratory characteristics than those with IgG MGUS. *Patients and methods:* We evaluated levels of hemoglobin (Hb), creatinine, PCR, B2-microglobulin, Immunoglobulins (Ig), FLC and K/L ratios in 27 pts with IgA MGUS and in 147 with IgG. FLC dosage was performed with Binding Site method (K/L normal range 0,23 - 1,65). *Results:* There are not significant differences about Hb values, total protein, renal function, PCR and B2 microglobulin. In patients with IgA MGUS, the value of monoclonal IG is often higher than the normal limit of IgG MGUS (81.7 *vs* 43.5%; p <0 <0003). This is due to small monoclonal bands are easily found in IgG MGUS. IgMs are the most frequently immunoglobulins reduced in both groups, but immunoparesis is present in 62.9% of IgA MGUS and in 31.4% of IgG (p-0.007). The value of uninvolved chains does not seem to differ between the two groups. The number of patients with monoclonal light chains higher than the upper limit is more frequently in IgA MGUS then IgG MGUS (73.1% *vs* 65.9%) but the difference is not significant. The number of patients with altered K/L is higher in IgA MGUS (59.2% *vs* 51%), but also this difference is not currently significant. The difference that seems to be significant even with a larger population than the previous controls, is that patients with simultaneous altered K/L and immunoparesis is more frequent in IgA group. In fact, in this group, both parameters are altered in 44.4% of cases and normal in 14.8%, while in IgG group are altered in 21.7% and normal in 39.4%. *Conclusions:* Patients with IgA MGUS show some laboratory characteristics different from patient with IgG MGUS. This could be compatible with more aggressiveness. It is important to evaluate if it's necessary to provide a closer follow up for this group of patients with simultaneous Immunoparesis and K/L alteration.

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BENDAMUSTINE-BORTEZOMIB-DEXAMETHASONE (BVD) IN THE MANAGEMENT OF RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A REAL-LIFE EXPERIENCE

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Bendamustine is a bifunctional alkylating agent, with low toxicity, proved to be effective in relapsed, refractory and in new diagnosed Multiple Myeloma (MM). It has been evaluated efficacy and tolerance of Bendamustine, in combination with bortezomib-dexamethasone (BVD) in patients with relapsed and refractory MM (rrMM), whose prognosis is particularly severe. A regional retrospective real-life analysis of patients with rrMM who had been treated with BVD as salvage therapy has been performed. 56 patients (31 M/25 F), with rrMM, median age at diagnosis 57.3 years (r. 36-82), median age at start of treatment 61.8 years (r.37-83) treated with several lines of treatments (median 6, r. 2-11), every refractory to all the drugs previously received (also Bortezomib), received BVD (Bendamustine 90 mg/sqm days 1,2; Bortezomib 1.3 mg/sqm days 1,4,8,11, Dexamethasone 20 mg days 1,2,4,5,8, 9,11,12, Pegfilgrastim day +4) every 28 days, until progression. ISS was equally distributed, and cytogenetic was evaluable in 12 patients, and in particular one del13q and one t(11;14). All the patients had previously been treated with schedule containing bortezomib and IMIDs, and 30% had also received radiotherapy. 67% of them had undergone at least to a single auSCT. All patients were relapsed and refractory to last therapies received before BVD. Bendamustine was well tolerated, with grade 3 transfusion-dependent anemia in 41% of patients, and 37% grade 3 neutropenia (no ospedalization was required, no septic shocks were observed). No severe extrahematologic toxicity was observed, only grade 1 gastrointestinal side effect (nausea), treated by common antiemetic drugs. According to IMWG, after a median follow-up of 14 months (r.2-36), ORR was 64% (36/56 : 4 CR, 7 VGPR, 16 PR, 9 MR) with 8 PD and 12 patients in SD, which can be considered as an impressive result in this subset of rrMM patients. In particular, for 11 patients, BVD was, after having achieved at least a PR, a bridge to second auSCT, and for two patients a bridge to alloSCT. Median time to response was 1.2 months (r.1-3), median OS from diagnosis was 62.7 months (range 6-151), median OS from start of Bendamustine was 9.8 months (range 2-36). BVD has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to all available therapeutic resources, and, in particular cases, it could be considered as a bridge to a second autologous or allogenic SCT.

Table 1.

Total patients	**56**
Male	31
Female	25
Median age, years	
at diagnosis, (range)	57.3 (36-82)
at start of BVD, (range)	61.8 (37-83)
Previous regimens	
median no. (range)	6 (2-11)
FISH analysis	**12/56**
negative	10
del13q	1
t(11;14)	1
Previous therapies : no. of patients/(%)	
Bortezomib	56 (100%)
IMIDs	56 (100%)
Autologous SCT	38 (67%)

P222

LENALIDOMIDE AT THE DOSE OF TWENTY-FIVE MG EVERY OTHER DAY IN PATIENTS AFFECTED BY MULTIPLE MYELOMA AND RENAL FAILURE : A REAL-LIFE EXPERIENCE

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With normal renal function, lenalidomide reaches its maximal plasma concentration after a median time of 0.6-1.5 h, and it is cleared by glomerular filtration and active tubular secretion in 3 to 4 hours. Serum half-life increases up to 9 hours if moderate/severe renal impairment (RI) is present (creatinine clearance <50 or <30 mL/min, respectively). In the latter cases a reduction of the daily dose is recommended. Dose adjustment based on RI severity decreases the daily amount of lenalidomide from 15 up to 5 mg (in patients undergoing dialysis); other studies include a schedule with 10 or 15 mg every other days. In this report, a retrospective experience on the administration of lenalidomide 25 mg every other day for patients with MM and RI is reported. From March 2014 to February 2016, 19 consecutive patients, 11 female and 8 male, with a median age of 63.3 years (r. 49-81) affected by advanced MM (median number of previous treatment lines: 3, range: 1-5, all including bortezomib) with concomitant renal failure not in dyalitic support (median ClCr 36.4 ml/min, range: 18-66) were treated, after informed consent, with monthly 21-day courses of 25 mg lenalidomide every other day and dexamethasone (20-40 mg on days 1-8-15-22, every 28 days). Disappearance of urinary light chain and reduction of serum creatinine (complete response) were detected in 7 patients (36.8%); 3 patients (15.7%) had a very good partial response, 3 (15,7%) had a partial response, 4 of them (21.0%) were in stable disease, whereas 2 patients (10.5%) had signs of progressive disease. Overall response ratio was 68.2%. More than half of the patients (11/19, 57.8%) had a renal response (median calculated ClCr 51.5ml/min, range 20-148). Median progression free survival was 8 months (range 3-18 months). No patient experienced grade 4 myelotoxicity; four patients required red cell transfusions for grade 3 anemia. No SAE occurred during treatment. Dose adjustment RI-related of Lenalidomide is recommended in most guidelines, but there is not a leading scheme with a proven effectiveness more than others. These preliminary observations point to a significant therapeutic effect of lenalidomide, at the dose of 25 mg every other day for 21 days, in more than half of a small population of patients with advanced MM and renal impairment, with not negligible logistic and economic advantages. However, these results should be validated by controlled studies involving larger number of patients.

P223

POMALIDOMIDE-DEXAMETHASONE IN THE MANAGEMENT OF HEAVILY PRETREATED MULTIPLE MYELOMA

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In this retrospective observational trial, It has been evaluated efficacy and tolerance of pomalidomide plus dexamethasone (PD) as salvage regimen in heavily pretreated patients with relapsed and refractory MM (rrMM), whose prognosis is particularly severe. 22 patients (13 M/9 F), with rrMM, median age at diagnosis 68 years (r. 54-80), and median age at start of treatment 71.5 years (r.61-36) treated with several lines of treatments (median 5, r. 2-8), every refractory to all the drugs previously received (also Bortezomib, Thalidomide and Lenalidomide), received PD (Pomalidomide 4 mg for 21 days, Dexamethasone 40 mg days 1,8,15,22, Pegfilgrastim day +8) every 28 days, until progression with ISS was equally distributed, and cytogenetic was evaluable in 12 patients, and in particular three del13q and one t(11;14) were present. All the patients had previously been treated with schedule containing bortezomib and IMIDs. 50% of them had undergone at least to a single ASCT. All patients were relapsed and refractory to last therapies received before PD. Pomalidomide was well tolerated, with grade 3 transfusion-dependent anemia in 45% of patients, 4% grade 3 neutropenia (pegfilgrastim in primary prophylaxis was given, no ospedalization was required, no septic shocks were observed), 18% grade 3 thrombocytopenia without hemorrhagic events and transfusion-dependence. No severe extrahematologic toxicity was observed. According to IMWG, after a median follow-up of 7.5 months (r.1-14), ORR1 (≥PR) was 39.1% (1 CR, 2 VGPR, 6 PR), but, considering that we are evaluating a cohort of heavily pretreated patients without any other alternative treatment, with really poor prognosis, another parameter should be considered, ORR2 (≥SD), considering stable disease as a successful result in progressive MM. ORR2 was 77% (1 CR, 2 VGPR, 6 PR, 8 SD). These can be considered as impressive result in this subset of rrMM patients. Oral treatment gives a really good compliance, in frail and unfit patients, and response, when present, is always really fast (median time to response: 2 months (r.1-11)), median OS from diagnosis was 84 months (range 27-228), median OS from start of pomalidomide was 8 months (range 1-14). PD has shown significant efficacy and a very good compliance,

thanks to oral administration, in a particularly severe setting of heavily pretreated patients, relapsed and refractory to all available therapeutic resources.

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BORTEZOMIB, LENALIDOMIDE AND DEXAMETHASONE IN THE MANAGEMENT OF RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A REAL-LIFE EXPERIENCE

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Bortezomib, Lenalidomide and Dexamethasone is one of the best option for frontline treatment. However, it can show interesting results also in relapsed and refractory patients, thanks to the synergestic effect of these agents. In this retrospective observational study, it has been evaluated the tolerability and efficacy of the combination of bortezomib plus lenalidomide plus dexamethasone (VRD) in patients with relapsed and refractory Multiple Myeloma (rrMM). 22 patients (16 M, 6 F), with rrMM, median age 66 years (M, range 38-74) and 59.5 years (F, range 54-69), had immunoglobilin (Ig) Gk MM (36.4%), IgG MM (27.3%), IgAk MM (13.6%), MM detected in urine only (13.6%) and non-secretory MM (9.1%). 36.3% of patients had ISS-1, 50% ISS-2 and 13.6% ISS-3; 3 patients had high cytogenetics risk with deletion of chromosome 13 (del13q). Patients were treated with the VRD regimen (Bortezomib 1.3mg/sqm days 1,4,8,11; Dexamethasone 20 mg days 1,2,4,5,8,9,11,12 and oral Lenalidomide 25 mg daily on days 1-21), with a median of 4 cycles (range 1-17). Patients had received 2.5 median (range 1-5) lines of therapy. 16 of them had been treated with schedules containing Bortezomib and Thalidomide in first line therapy, 1 had been treated with alkylating agents and Bortezomib, 2 treated with Melphalan and Prednisone. 11 of them had undergone to autologous SCT. 15 patients received VRD in second line, 6 patients in third line and 1 in fifth line. According to IMWG, ORR was 77.2% (17/22: 4.5% CR, 45.4% PR, 27.3% SD, 22.73% PD). Median time to response was 3 months (range 1-23), median OS from diagnosis was 48 months (range 12-214). Considering safety, VRD was well tolerated, with grade 1 anemia in 3 patients and grade 2 anemia in 1 patient successfully managed with ESAs, and thanks to the way of administration, also compliance is good. Bortezomib-lenalidomide-dexamethasone regimen, thanks to a notable proved synergistic mechanism of action between bortezomib and lenalidomide, had shown significant efficacy in severe setting of heavily pretreated patients, relapsed and refractory to bortezomib and lenalidomide.

Allogeneic and Autologous Transplantation 1

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MONOCENTRIC OBSERVATIONAL STUDY ON PATIENTS AFFECTED BY SECONDARY HAEMATOLOGICAL NEOPLASIA (T-HN) SUBMITTED TO ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Therapy related hematological neoplasia (t-HN) occur due to direct mutational events of chemotherapeutic agents and radiotherapy. Disease latency, mutational events and prognosis vary with drugs categories. *Materials and Methods:* we describe a cohort of 33 patients, 21 females and 12 males, with median age of 53 years (range, 20 to 64), submitted to allogeneic stem cell transplantation (SCT) in our department between September 1999 and March 2017. Patients had a history of solid tumor in 17 cases (thyroid n=2, breast n=10, CNS n=1, bone n=1, bladder n=2, anus n=1), hematological disease in 15 cases (HL n=2, ALL n=1, CLL n=2, NHL n=9, AML n=1), and both of them in one case (breast and NHL). All but one received previous treatment (chemotherapy n=20, radiotherapy n=5, combined chemo/radio n=7). After a median of 36 months (range 1-190) from first neoplasia, patients developed t-AML (n=20), t-Ph+ ALL (n=2), or t-MDS (n=11). Among 30 evaluable patients, 19 had karyotype abnormalities (poor n=11, intermediate n=6, good n=2), while 11 showed a normal one. Patients received conventional chemotherapy in 15 cases, 5-azacytidine in 11 cases, combined chemo/AZA in one case and TKI in another one, whereas 5 patients proceeded immediately to SCT. Median HCT-CI was of 3 (range, 3 to 9). Conditioning was MAC in 22 cases and RIC in the others, with stem cell obtained from peripheral blood in 26 cases, bone marrow in 6 cases and cord blood in another one. Donor was related in 19 cases and MUD in the others. Fourteen patients had achieved a complete disease remission (CR) before SCT. *Results:* five patients died early after SCT due to transplant-related mortality (TRM). Twenty-five (89%) patients obtained a CR at bone marrow examination performed on day 30 after SCT. Nineteen of them (68%) maintained a sustained CR, while 6 patients relapsed after a median time of 7 months (range, 3 to 15). At the follow up time (March 2017) 16 patients were alive with a median OS of 40.5 months (range 1-193), while 17 patients died after a median of 4 months (range 0.3-27) by relapse mortality in 5 cases and non-relapse mortality in the other 12 patients (GvHD n=3, PTLD n=1, infection n=8). *Conclusions:* Global OS was 48% and after SCT 68% of patients with t-HN obtained a prolonged and sustained complete remission of the underlying disease. Figure 1.

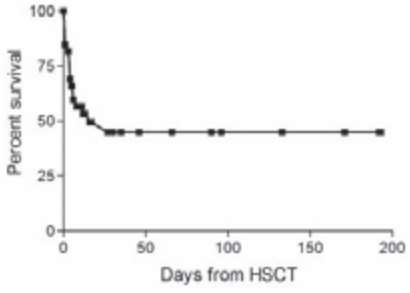

Figure 1. OS of secondary neoplasia after SCT.

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DONOR-SPECIFIC ANTI-HLA ANTIBODIES AND PRIMARY GRAFT FAILURE RISK IN MISMATCHED HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Introduction: Detection of donor-specific anti-HLA antibodies (DSA) has been reported to be associated with graft failure (GF) in mismatched SCT, but their frequency and their clinical impact remain unclear. *Methods:* We prospectively evaluated the presence of DSA, using Solid Phase system, Luminex commercial kits, in adult patients undergoing unmanipulated unrelated SCT mismatched for 1 antigen or allele in HLA class 1 (USCT) and unmanipulated haploidentical SCT (HaploSCT). DSA binding level was expressed as mean fluorescence intensity (MFI). *Results:* 98 patients underwent allogeneic SCT in our department between May 2015 and March 2017. Forty-one/98 underwent mismatched SCT: 22/41 (54%) USCT and 19/41 (46%) HaploSCT. USCT conditioning was BuCy (5 pts) or Fluda-based (17 pts) with CyA, ATG and MTX as GvHD prophylaxis. HaploSCT conditioning was TBF with CyA, Mycophenolate and post-transplant Cy as GvHD prophylaxis. DSA were detected in 7/41 pts (17%): 3/22 USCT (14%) and 4/19 HaploSCT (21%). Median MFI was 1160 (765 – 22400), with 3 pts having MFI>2000. The first 2 pts of our series with DSA (MFI 10500 and 22400 respectively) did not receive desensitization treatment before transplant and failed to engraft. One untreated patient died of septic shock before engraftment. The other 4 patients received a desensitization treatment to improve engrafment, according to the DSA MFI level. Two pts with DSA MFI level of 1300 and 1160 respectively received 2 pre-transplant Plasma Exchange procedures (PEX) and both engrafted. We used a desensitization treatment based on 4 PEX, intravenous immunoglobulin (1 g/kg) and Rituximab (375 mg/sm) in 2 pts with high levels of DSA. One of these pts had DSA MFI 900 and experienced primary GF with increasing DSA levels (maximum MFI 10500) after reinfusion of stem cells; so a second transplant from the same donor was performed after desensitization treatment. The second pts received the desensitization procedure before the first transplant. Both of them experienced DSA disappearance and achieved engraftment. *Conclusions:* DSA were detected in mismatched USCT candidates (14%) and in HaploSCT candidates (21%) and they were associated with GF in 3 pts who did not receive any desensitization treatment before stem cell reinfusion. Employing a desensitization treatment according the DSA levels achieved DSA clearance and engrafment in all the 4 pts in which it was performed, underlining the potential benefit of the procedure in this setting.

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EXTRACELLULAR VESICLES AS POTENTIAL BIOMARKERS OF GRAFT-VERSUS-HOST-DISEASE

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Graft-*vs*.-Host Disease (GVHD) is a cause of toxicity and mortality after allografting. Reliable, non-invasive, predictive biomarkers may be a major advance to improve outcomes. No current validated test has reliably become predictive of GHVD onset or its treatment response. Extracellular Vesicles (EVs) recently emerged as a promising new category of biomarkers in different scenarios including inflammation, tissue damage and cancer. EVs can be easily extracted from biological fluids such as blood and urine, making them attractive for diagnostic applications. The aim of this explorative study was to investigate a potential correlation between GVHD and surface antigens on EVs. We selected a homogeneous patient population with myeloma, transplanted with mobilized peripheral blood, and mostly (90%) prepared with a reduced-intensity conditioning. In fact, biomarkers may be influenced by factors including age, disease, conditioning, and all causes of tissue and/or endothelial inflammation. Forty-one patients were enrolled. Serum samples were prospectively collected before and monthly after transplant up to 6 months. After extraction, EVs were characterized by flow-cytometry with a panel of antibodies against specific membrane proteins potentially predictive of GVHD (CD44, CD138, CD146, KRT18, CD120a, CD8, CD30, CD106, CD25, CD31, CD144, CD86, and CD140a). At given time-points, total EVs concentration, fluorescence distribution and percentage of positive EVs for each marker were determined. Significant correlation between 3 potential biomarkers and acute GVHD onset was observed by both logistic regression analysis and Cox proportional hazard model. CD146 (MCAM-1) was correlated with an increased risk (by almost 60%) of developing GVHD, whereas CD31 and CD140a (PECAM-1 and PDGFR-alpha) with a decreased risk (by almost 40% and 60%, respectively). All 3 biomarkers showed a proportional change in signal level from baseline up to the onset of GVHD. No statistically significant association between biomarkers and chronic GVHD was seen. CD146 and CD31 belong to the Cell Adhesion Molecule family (MCAM-1, and PECAM-1, respectively) which are crucial for endothelium and immune cells interaction. CD140a is the PDGFR-alpha, which is involved in angiogenesis, fibroblast migration and wound healing. All these proteins may play a role in acute GVHD pathogenesis. Our novel study encourages future investigations into the potential correlation between EVs and acute GVHD.

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THE INCREASED EXPRESSION OF COLLAGEN IN CGVHD FIBROBLAST IS STRONGLY INHIBITED BY THERAPEUTIC CONCENTRATIONS OF NILOTINIB, WHICH SWITCHED OFF THE TGF SIGNALING, VIA P-SMAD2 DOWN-REGULATION

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Introduction: Dermal fibrosis and sclerosis are pathologic features of Scleroderma-like chronic graft-*versus*-host disease (Scl-cGVHD).Targeted therapy with tyrosine kinase inhibitors (TKI) demonstrated clinical efficacy in Scl-cGVHD;however, the molecular basis underpinning the clinical effects are not fully elucidated. In this study we investigated a potential terapeutical target of the dermal cGVHD pathophysiology by studying the molecular features of pathological skin fibroblasts (GVHD-Fbs) and the efficacy of Nilotinib in modulating fibrosis. *Materials and Methods:* Primary fibroblast cultures (GVHD-Fbs) were obtained from affected skin biopsies from 5 patients with active cGVHD whereas Human Dermal Fibroblasts adult (n-Fbs) were used as control. In subsets of experiments,after starvation, n-Fbs and GVHD-Fbs were stimulated with 10ng/ml TGF or 1μM of Nilotinib for 48h.The concentration of Nilotinib has been chosen according to its plasma levels achieved in cGVHD patients receiving the drug in a concurrent phase 1-2 study. The expression of COL1 1, COL1 2 and the activation of TGF signaling (p-Smad2) was assessed by western blot (WB) analysis. The localization of COL1 1, COL1 2 and p-Smad2 in primary cells were performed by immunofluorescence staining (IF). *Results:* Gene expression in RT-PCR of COL1 1 and COL1 2 in GVHD-Fbs has been previously reported at the last ASH 2016, and was respectively 4 and 1,6 fold higher compared to n-Fbs (p=0.02). As expected,preliminary also WB analysis confirmed an increased expression of collagen in GVHD-Fbs compared to n-Fbs.Moreover, incubation with TGF increased collagen expression in n-Fbs, but not in GVHD-Fbs.After incubation with escalating concentrations of Nilotinib, we found that therapeutic doses of this drug (≥1μM) were able to reduce expression of COL1 1 and COL1 2 protein.At the same time we showed a "switch off" of TGF signaling, resulting in a reduction of p-Smad2 expression in WB analysis and a reduction of nuclear staining in IF.(Figure 1). *Conclusions:* In a previous work we demonstrated that GVHD-Fbs are characterized by exaggerated mRNA collagen expression, effectively inhibited by therapeutic concentration of Nilotinib. Now our data show that the increased collagen production in GVHD-Fb, mediated by the p-Smad2 pathway activation, is strongly inhibited by therapeutic concentrations of Nilotinib,

suggesting a pivotal role of this drug in inhibiting the fibrotic process via a TGF -dependent mechanism in cGVHD patients.

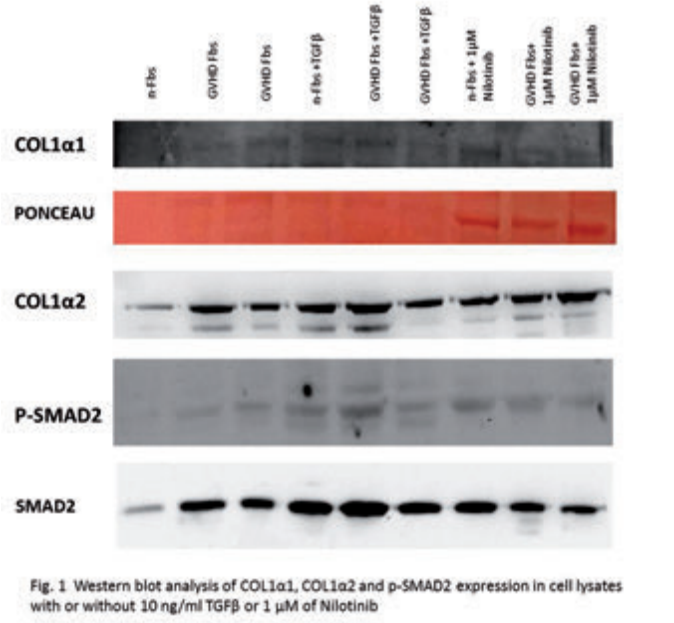

Fig. 1 Western blot analysis of COL1α1, COL1α2 and p-SMAD2 expression in cell lysates with or without 10 ng/ml TGFβ or 1 μM of Nilotinib

Figure 1.

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CRYOPRESERVATION DOES NOT REDUCE THE IN VITRO INHIBITORY FUNCTION OF GMP- ISOLATED HEALTHY REGULATORY T CELLS

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Using TREGs in immunotherapy may be beneficial in several immune mediated diseases, as Graft *Versus* Host Disease (GVHD), through induction of immunologic tolerance. The possibility of cryopreserving TREGs might lead to the administration of multiple doses at different time point, thus potentially increasing their efficacy in chronic diseases. However, there are few and controversial data on the functionality of TREGs after cryopreservation. Here, we evaluated the phenotype and the inhibitory capacity of thawed TREGs. TREGs were purified from leukapheresis of normal donors (N=3) by double immunomagnetic depletion (CD8 and CD19) followed by CD25 enrichment using the CliniMACS system (Miltenyi Biotec) under GMP condition. The cells were cryopreserved in saline solution containing 10% Human Serum Albumin (HSA) and 10% DMSO with a controlled-rate freezing. Cell viability was assessed by 7-AAD staining. Number/phenotype and function were evaluated on fresh and thawed TREGs. Cryopreserved autologous T effector (Teff) cells were used in MLR assays. Before cryopreservation the TREGs enriched product mean viability was 95±4% and the mean percentage of CD45+CD4+CD25+CD127low and CD45+CD4+CD25+CD127lowFoxP3+ cells was 74±13% and 66±10%, respectively. We then analysed the TREGs enriched product after thawing. Mean viability of thawed TREGs, by 7-AAD staining, was 85±7%. The viable TREGs were almost totally CD4+CD25+ (97±2%). The mean percentage of CD4+CD25+CD127low and CD4+CD25+CD127lowFoxP3+ thawed cells was 73±14% and 71±20% respectively. The contaminant cells present in the TREG enriched product were mostly CD4+CD25+CD127+ (around 18%). We further characterized the phenotype of the CD4+CD25+CD127low population. This population was almost totally Foxp3+ (93±6%) and expressed selected markers at various degree (CD62L (50±2%), CD15s (6±2%), CD45RA+ (19±3%), HLA-DR+ (15±10%), CCR7+ (74±5%), CD49d (52±14%), CD26+ (1±0.4%), CD196+CD161+ (4±1%). Notably, viable thawed TREGs were able to induce inhibition of autologous Teff cells in a 1:2 Tregs:Teff ratio as freshly isolated TREGs: 44±16% (thawed) *vs* 55±24% (fresh) of inhibiton (p>0.1). In conclusion, here we demonstrated that thawed TREGs from healthy donors mantain a stable phenotype. In addition, in our hands TREGs show good suppressive ability after thawing despite lower expression of CD62L and CD15s (markers of most suppressive TREGs) as compared with the available published data (Florek et al 2015; Miyara et al 2015).

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COMPARATIVE ANALYSIS OF IMMUNE RECONSTITUTION SHOWS BETTER RECOVERY OF B, NK AND PLASMACYTOID DENDRITIC CELLS AFTER ALLOGENEIC CORD BLOOD AS COMPARED TO PHERIPHERAL BLOOD STEM CELL AND BONE MARROW TRANSPLANTATION

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Introduction: The reconstitution of different immune cell subsets after hematopoietic stem cell transplantation (HSCT) occurs at different time points and different factors can contribute to delay this reconstitution. We have studied the impact of several factors on immune reconstitution (type of disease, conditioning regiment therapy, type of stem cell source, Graft *Versus* Host Disease (GVHD) and drug therapy used). *Patients and methods:* We have studied 503 adult patients receiving pheripheral blood stem cell (PBSC)(n=262), bone marrow (BM)(n=191) or cord blood (CB) (n=48) transplant at the S. Orsola Hospital. Data were collected between 2000 and 2014. We have analysed the immune cell subsets in the peripheral blood at specific time points from 1 month up to 1 year after transplant. A total of 1368 samples were processed. Multicolor flow cytometry was employed to measure the numbers of circulating T and B lymphocytes, NK cells and APC subtypes (monocytes, plasmacytoid (pDC) and mieloid (mDC) dendritic cells). *Results:* The recovery of pDC at 3 through 12 months after transplant was significantly increased in patients receiving CB as compared to both PBSC and BM transplants [7x10e6/l (2.4-11.7) at 3 months after transplant in CB recipients *vs* 3x10e6/l (1.3-5.7) and 2.9x10e6/l (1.3-5.7) respectively in PBSC and BM recipients] (p 0,0001). Moreover we have confirmed the increase of B and NK cell recovery and the delay in T cell recovery in CB transplants. The only other factor affecting immune recovery was the chronic GVHD. The number of pDC was reduced in patients with extensive cGVHD compared to patients with limited GVHD or without cGVHD at 3 months [1.9 x10e6/l (0.6-4.1) *vs* 4.1 x10e6/l (2-7.1) and 2.5 (1.1- 5) respectively](p<0,001) up to 1 year. The recovery of T cells and NK cells was unaffected by cGVHD, whereas the B cell recovery was also reduced by cGVHD (significative value only at 9 months). *Conclusions:* Our study shows that patients undergoing CB transplantation have better reconstitution of plasmacytoid DC, as well as NK cells and B cells, as compared to patients receiving PBSC and BM grafts. Furthermore, pDC recovery was significantly delayed in patients with cGVHD.

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A COMPARATIVE ANALYSIS OF BIOSIMILAR VS ORIGINATOR FILGRASTIM IN COMBINATION WITH PLERIXAFOR FOR STEM CELL MOBILIZATION IN LYMPHOMA AND MULTIPLE MYELOMA; A PROPENSITY SCORE-WEIGHTED MULTICENTER SURVEY

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Background: Autologous transplantation (auto-SCT) is a mainstay of treatment for many malignant and non-malignant diseases. Biosimilar G-CSFs are today widely employed as part of mobilization strategies; however, little data is currently available about the relative efficacy of biosimilar G-CSFs when combined with the CXCR4 antagonist plerixafor, as compared to originator G-CSF. *Patients and methods:* We performed a retrospective, propensity score weighted analysisof patients affected by lymphoma or multiple myeloma undergoing PBSC collection at 23 italian centers from January 2008 to December 2016, mobilized with plerixafor combined with either biosimilar (Zarzio®, Sandoz Industrial Products; Tevagrastim®, Teva Pharmaceutical Industries) or

originator filgrastim (Neupogen®, Amgen). *Results:* A total of 296 patients fulfilled the inclusion criteria. Patients receiving BIO+PLX combination were more likely to exceed the PB-CD34+ threshold of 5/mcl and 20/mcl before and after plerixafor administration, respectively, as compared to the OR+PLX group (weighted OR=3.6; robust 95% CI 1.5–8.4; weighted OR=6.8; robust 95% CI 2.6–17.6). Further, patient mobilized with the BIO+PLX combination showed higher probability of collectinga stem cell dose of 2x106 CD34+/Kg or higher (weighted OR=6.1; robust 95% CI 1.9-18.9). No significant differences were observed between the two study cohorts in probability of achievement of target stem cell dose (5x106 CD34+/Kg) and minimum and target stem cell dose for double transplant in multiple myeloma patients (4 and 10x106 CD34+/Kg, respectively). Finally, patients who received BIO+PLX were more likely to reach the apheresis procedure as compared to OR+PLX group (weighted OR=5.3; robust 95% CI 1.1-26.4). *Conclusions:* The combination of biosimilar filgrastim and plerixafor appears to be equally and even more effective as compared to originator and plerixafor for stem cell mobilization in patients at high risk of mobilization failure. This data strongly support standard inclusion of biosimilar filgrastim in mobilizing protocols even in the challenging setting of poor mobilizers, as significant cost saving seems to be accompanied by strong efficacy.

Table 1. Stem cell mobilization kinetics.

Characteristic	Originator N = 197 (66.55%)	Biosimilar N = 99 (33.45%)	P Value
PB-CD34+ before PLX	5.3 (2.0-9.0)	10.0 (6.0-16.0)	<0.001
PB-CD34+before PLX>5/mcl	51%	77%	<0.001
PB-CD34+ after PLX	24.0 (12.0-48.0)	50.2 (30.0-71.0)	<0.001
PB-CD34+ after PLX>20/mcl	56%	88%	<0.001
CD34+/Kg collected	4.0 (2.5-6.8)	4.2 (3.0-5.9)	0.345
CD34+/Kg collected>2x10⁶	82%	92%	0.026

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MODIFIED BEAM SCHEDULE WITH LOMUSTINE INSTEAD OF CARMUSTINE IN THE CONDITIONING REGIMEN OF AUTOLOGOUS STEM CELL TRANSPLANTATION IN LYMPHOMA PATIENTS: ANALYSIS OF EFFICACY AND TOXICITY

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Background: The treatment of choice for relapsed/refractory lymphoma consists of high dose chemotherapy (HDC) followed by autologous stem cell transplantation (ASCT). In our Institution the classical BEAM conditioning regimen was modified by using Lomustine (CCNU) in place of Carmustine (BCNU). *Methods:* Between 2009 and 2016, 90 lymphomas (79 Non-Hodgkin Lymphoma and 11 Hodgkin Lymphoma) patients (pts) were treated with CEAM regimen followed by ASCT. The CEAM regimen consisted of: CCNU 200 mg/m^2 on day -6; Etoposide 200mg/m^2 (total dose 800mg/m^2) on days -5 to -2; Cytarabine 400mg/m^2 (total dose 1600mg/m^2) on days -5 to -2; Melphalan 140mg/m^2 on day -1. Peripheral Blood Stem Cells (PBSC) were infused on day 0. *Results:* The median duration of hospitalization was 20 days (range 16-57). No pts died during hospitalization. The median number of CD34+ cells infused was 5.22x10^6/kg (range 1.72-15.35). Median time to haemopoietic engraftment (neutrophilic one when more than 500/mmc) occurred on day 16 (range 7-56). Fever of unknown origin during neutropenia occurred in 82 patients (91%), whereas etiology was defined in 10 pts (11%) and only 2 had a bacterial pneumonia. Gastrointestinal mucositis was observed in 81 pts (90%), whose 47 had grade I-II (52.3%), 30 grade III (33.3%) and 4 pts grade IV (4.4%). Median follow-up was 3.56 years (yrs) (range 1.02-7.39); median overall Survival (OS) was not reached at 5 yrs; besides, median event-free survival (EFS) was reached at 4.06 yrs. Transplant-related mortality at +100 was 0%. Finally 25 pts (25.5%) died due to disease progression. *Conclusions:* In this series we found that CEAM schedule is well tolerated without significant regimen-related complications in pts with sensitive disease. No pts died of TRM at +100. When compared to the most widely used BEAM regimen, the results of CEAM seem neither to add significant toxicity nor to be inferior in terms of clinical outcomes. Therefore we can conclude that Lomustine can be a valid alternative to Carmustine as conditioning regimen for ASCT in lymphoma pts without losing clinical effectiveness. Anyway, further strategies should be investigated for pts with refractory lymphomas.

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ECONOMICS OF HEMATOPOIETIC CELL TRANSPLANTATION: ROLE OF ACUTE AND CHRONIC GVHD

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In the past 20 years, a remarkable increase in number of allogeneic hematopoietic stem cell transplants (HSCT) was registered. Much has been done to reduce adverse events; however, graft *versus* host disease (GvHD) still represents a major cause of morbidity and mortality. A comprehensive costs evaluation of HSCT is lacking. GvHD is a major determinant of economic consequences of HSCT and clinical decision-making processes including coast evaluations are needed. To study this aspect, we have retrospectively selected 15 representative patients (pts) who underwent HSCT at our center between '09-'15: 5 pts (Group A) never experienced GvHD, 5 pts were diagnosed with chronic GvHD (Group B) and 5 with acute GvHD (Group C).

Table 1. Per group evaluation of factors relevant for direct costs in HSCT.

	Group A no GvHD n5	Group B chronic GvHD n5	Group C acute GvHD n5	Group B + Group C	
				Group D GvHD resolved n 5	Group E resistant GvHD n5
median follow-up (r)	1148 (1306-2532)	1812 (1107-1833)	1200 (254-1926)	1812 (1107-1926)	1279 (254-1829)
median days of hospitalization (r)	3 (0-25)	8 (0-37)	46 (0-230)	20 (0-46)	37 (0-230)
median n of day-hospital evaluation (r)	1 (0-12)	2 (1-12)	9 (0-24)	1 (1-15)	8 (2-24)
median n of outpatient evaluation (r)	32 (14-52)	50 (47-132)	53 (39-58)	50 (47-58)	88 (39-132)
median time to stop CsA / rapamycin	180	349	205	349	328
median time to withdrawal of all immunosuppressive drugs	180 (5/5)	699 (3 pts evaluable, 2 pts under treatment)	312 (2 pts evaluable, 2 pts under treatment for chronic GvHD, 1 pt dead due to acute GvHD)	420	n.a. (4 pts under treatment for chronic GvHD, 1 pt dead due to acute GvHD)
median n of acute GvHD treatments	n.a.	1 (0-1)	3 (1-5)	2 (1-4)	9 (5-15)
median n of chronic GvHD treatments	n.a.	3 (1-15)	6 (1-7)		
median time on 3rd generation anti fungal therapy (r)	219 (90-435)	570 (49-1406)	374 (83-907)	374 (83-717)	559 (49-1406)
median n of azole TDM evaluation (r)	17 (13-19)	24 (22-86)	31 (4-45)	22 (4-31)	36 (18-86)
absolute n of documented IFI	0/5	0/5	2/5	0/5	2/5)
median n of viral monitoring: CMV / EBV / HHV6	52 / 33 / 3	60 / 45 / 10	79 / 57 / 39	60 / 45 / 18	107 / 63 / 53
median n of viral reactivation requiring treatment (r)	1 (0-4)	1 (1-11)	2 (1-3)	1 (1-2)	3 (1-11)
median n of days of treatment for CMV: Ganciclovir and Valganciclovir / Foscarnet	27 / 13	28 / 4	21 / 14	26 / 12	30 / 0
median n of CT scan evaluation (r)	1 (0-4)	3 (3-12)	5 (2-9)	3 (2-3)	8 (6-12)
median n of endoscopic evaluation (r)	0 (0-1)	0 (0-0)	1 (0-6)	0 (0-1)	0 (0-6)
median n of red blood cells / platelets transfusion (r)	0 (0/0)	0 (0-2) / 0 (0-0)	3 (0-79) / 4 (0-105)	0 (0-0) / 0 (0-1)	3 (0-79) / 4 (0-105)

The groups were homogeneous in term of conditioning regimens, prophylaxis, grafts and donor selection according di Institutional policy as stated in JACIE standards. Most common diagnosis was acute myeloid leukemia (10/15). Five pts were transplanted from unrelated donor, 4 from matched siblings and 6 from haploidentical donors. Five pts received cyclosporine-based prophylaxis, 10 pts received sirolimus-based prophylaxis. We monitored several key parameters starting after GvHD diagnosis or after discharge for HSCT procedure, whichever occurred first. Factors relevant for costs are reported in Table 1. We documented lower hospitalization needs, fewer outpatient evaluations, shorter time of immunosuppressive treatment, lower incidence of infections and need of anti-infective treatment in Group A *versus* Group B and Group C. Differences are even clearer if patients from Group B and Group C are subdivided in Group D (complete resolution of GvHD and withdrawal of immunosuppressive treatments) and Group E (steroid resistant GvHD): pts in Group E are more likely to undergo re-hospitalization, to accede more frequently to the outpatient clinic, to take more ancillary therapies. Our results indicate that pts with GvHD are more exposed to complications and medicalizations, with worst performance in pts who required more than 2 therapies lines; this translates into higher burden of direct costs. Previous studies on cost analysis of pts who underwent HSCT showed that costs were highest during the first year. In our opinion, it is crucial to monitor all these key factors in a long-term perspective. Impact of new therapies for GvHD prevention should be balanced according to direct costs evaluation in patients experiencing severe acute and chronic GvHD.

P234

RESIDUAL RED BLOOD CELLS IN ABO-INCOMPATIBLE BONE MARROW TRANSPLANTS: DETRIMENTAL EFFECT OF HIGHLY STRINGENT DEPLETION

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Major ABO incompatible bone marrow transplantations (BMT) require a stringent red blood cell (RBC) depletion of bone marrow (BM) grafts to prevent massive hemolysis in recipients. Although there are no specific guidelines, a maximum RBC residue of 30 ml (approximately 0.42 ml/kg for a person of 70 Kg body weight) is generally advised for adult patients (pts). Moreover, anti-A or anti-B hemagglutinin titers in recipients can be reduced by plasmapheresis or immuno-adsorption before the graft infusion. In order to critically review our current procedures, we analyzed hemolysis parameters, transfusion requirements, BM graft cell doses and times to engraftments in BMTs performed at our Institution between 2012 and 2017. Overall, 50 BMTs were analyzed: 28 pts received RBC-replete ABO-matched grafts, while 22 pts (17 major and 1 bidirectional ABO-incompatible BMT pts, and 4 ABO-matched BMT pts) received RBC-depleted grafts.

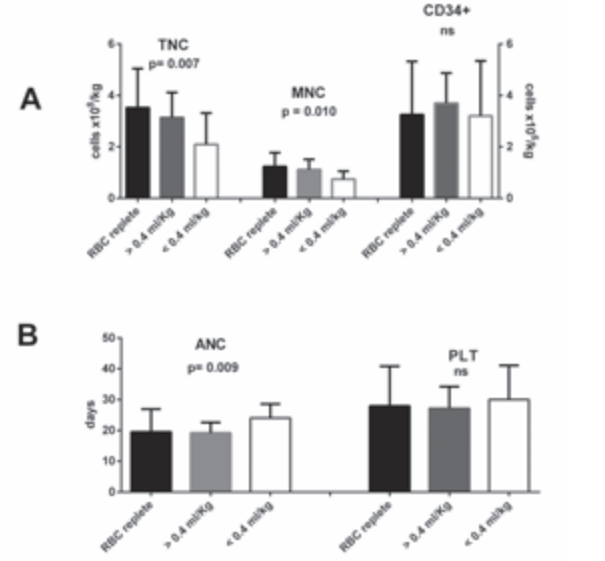

Figure 1.

According to the period, the RBC depletion was carried out by manual (succinyl-gelatine) or automated (Fresenius Kabi, Sepax, Biosafe) sedimentation. The median age was 50 years (range 17-66); 25 were males and 25 females. The anti-A and or anti-B hemagglutinin titres before BMT was recorded in 11 out of 18 AB0-incompatible BMTs. The median RBC graft content was 4.3ml/kg (range 0.86-8.91) in 28 RBC-replete grafts and 0.05ml/kg (range 0.05-1.39) in RBC-depleted grafts. No clinically relevant hemolytic transfusion reactions were recorded among AB0-incompatible transplants, independently of the RBC residues (higher or lower than 0.42ml/kg) or hemagglutinin titres (higher than 1:32). A slight increase of both LDH and bilirubin was observed in ABO-incompatible BMTs (data not shown). Moreover, pts receiving ABO-incompatible BMT required more RBC transfusions at 1 (p=0.009) and 6 months (p=0.001) than others. On the whole, pts receiving more stringently RBC-depleted grafts (*i.e.* less than 0.42ml/Kg of RBCs; N=14, 10 ABO-incompatible and 4 ABO-matched BMTs), received significantly less total nucleated cells and mononuclear cells than pts receiving both RBC-replete grafts (N=28) and less stringently RBC-depleted grafts (N=8) (Fig 1A); accordingly, they showed longer times for neutrophil engraftment (Figure1B). Overall, these findings suggest that less stringent RBC depletion in ABO-incompatible BMT may be safely accomplished, allowing to restrain the loss of hematopoietic cells.

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FIRST-LINE AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IS FEASIBLE AND EFFECTIVE IN ELDERLY MULTIPLE MYELOMA (MM) PATIENTS AND MEL200 SHOULD BE THE PREFERRED CONDITIONING REGIMEN. AN ANALYSIS OF 78 PTS TRANSPLANTED AT A SINGLE INSTITUTION

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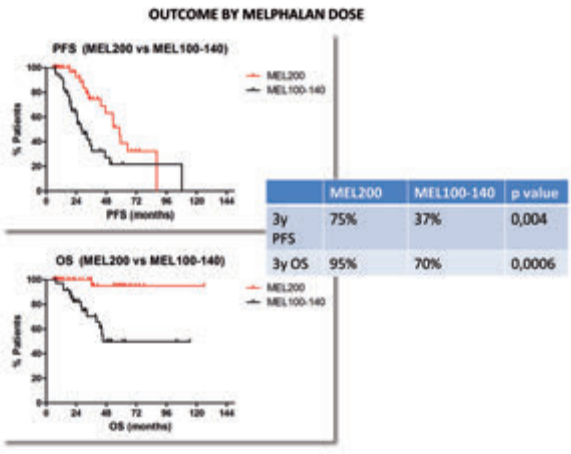
Spedali Civili

Background: The efficacy and safety of autologous stem cell transplantation (ASCT) in patients (pts) aged >65 with Multiple Myeloma (MM) is currently debated. A number of non comparative studies have shown acceptable toxicity and good results in selected elderly pts. Institutional policy at our center is to offer ASCT to pts with normal pulmonary and cardiac function and no major comorbidities. *Aims:* We report the results of first-line ASCT performed in consecutive patients aged 65 to 75 years at our Institution. The impact of potential prognostic parameters on outcome were analyzed. *Patients and Methods:* Between 2008 and 2016, 78 newly diagnosed MM pts aged ≥ 65 received ASCT as part of first-line treatment after VAD (19%) or bortezomib-based (81%) induction. They represented the 23% of newly diagnosed MM aged ≥ 65. Melphalan 200mg/m^2 (MEL200) conditioning was given to 55%, MEL140 to 8% and MEL100 to 37% of pts. The impact of age, pretransplant disease status (DS), ECOG PS, HCT-CI score and MEL dosage on progression-free (PFS) and overall survival (OS) were analyzed.



	MEL200	MEL100-140	p value
3y PFS	75%	37%	0,004
3y OS	95%	70%	0,0006

Figure 1.

Results: Complete Remission rate was improved from 5% before, to 41% after ASCT; Very Good Partial Response was stable at 32%. Grade

4 non-hematologic toxicity was observed in 12 pts (15%). Median hospital stay was 18 days, irrespective of age. Transplant related mortality (TRM) was 0%. Death rate was 19%; it was related to MM progression in 14/15 cases (93%). At the median follow-up of 3 years, PFS was 53% and OS 85%. At univariate analysis age < or ≥ 70 years impacted on PFS. PS 0-1 *vs* ≥2 negatively affected OS (88% *vs* 65%, p 0.013) but not PFS. Both HCT-CI risk score and pretransplant DS didn't influence outcome. A significantly better outcome was seen in patients receiving MEL200 conditioning, compared to lower MEL doses (PFS 75% *vs* 37%, p 0.004; OS 95% *vs* 70%, p 0.0006). At multivariate analysis, MEL200 remained the only independent prognostic factor both for PFS (p 0.012) and OS (p 0.021). *Conclusions:* ASCT was feasible and effective in many MM patients aged ≥65 and in selected patients aged ≥70 years, with manageable toxicity and no TRM. CR rate was significantly improved with ASCT. Neither HCT-CI score, age or DS were predictors of TRM. Age affected PFS and PS ≥2 affected OS, while HCT-CI score and pretransplant DS didn't affect the survival. Patients deemed eligible to ASCT should receive full-dose MEL200 which was the most important factor associated with better PFS and OS.

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RATE OF RE-ADMISSION IN PATIENTS UNDERGOING ALLOGENEIC TRANSPLANTS FROM IDENTICAL SIBLINGS, UNRELATED DONORS OR HAPLOIDENTICAL DONORS

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HLA identical siblings (SIB), unrelated donors (UD) and family HLA haploidentical donors (HAPLO) are currently being used for patients undergoing an allogeneic transplant (HSCT) for hematologic disorders. The outcome of these three different platforms is usually measured in terms of GvHD, non relapse mortality and survival, but days of admission and re-admissions are important in terms of morbidity and costs even though are usually not reported. We retrospectively analyzed 151 patients who received HSCT from February 2012 to August 2016 in our Department. Patients characteristics are shown in table 1. Relapses were excluded from the re-admission analysis.

Table 1. Characterists of patients and hospitalization.

	Total	Matched unrelated donor	Identical sibling Donor	Haploidentical relative donor
Number of patients	151	61	54	36
Male/female	86/65 (57/43%)	39/22(64/36%)	27/27(50/50%)	20/16(56/44%)
Median age at transplant	48 (range20-71)	45 (range19-66)	51 (range21-63)	50 (range22-71)
Disease				
AML	84(56%)	31(51%)	30(56%)	23(64%)
ALL	16(11%)	7(12%)	3(5%)	6(17%)
MDS	21 (14%)	10(16%)	7(13%)	4(11%)
NPM	8(5%)	5(8%)	2(4%)	1(3%)
Lymph	15(10%)	5(8%)	8(15%)	2(5%)
MM	7(4%)	3(5%)	4(7%)	
Time to PMN>500/mmc	17 days (range 9-90)	17 days (range 9-90)	17days (range10 -46)	21days (range18 -34)
Days from tranplant to discharge	23 days (range 7-90)	25 days (range 10-90)	21 days (range 10-47)	27 days (range 16-71)
Second hospitalization	51(34%)	28(46%)	13(24%)	10(28%)
Days of re-hospitalization	10(2-49)	10.5(4-49)	8(2-39)	12(2-39)

The median time from the transplant to discharge was 25 days for UD, 27 for HAPLO and 21 days for SIB: there was no significant difference between HAPLO *vs* UD (p=0.6), whereas the admission of both HAPLO and UD was longer than SIBS (p<0.01). Fiftyone patient out of 151 required of a new admission for complications after tranplant (28 out of 61 after MUD (46%), 13 out of 54 (24%) using a sibling donor, 10 out of 36 using an haploidentical donor (28%). There were significantly more re-admissions in UD *vs* SIB group (0.01) and a trend for more UD re-admissions *vs* HAPLO (p=0.08); siblings had the lowest number of readmissions. Time to neutrophil engraftment was comparable in HAPLO *vs* UD patients (p=0.1) and in SIB *vs* UD (p=0.1); the time was longer in HAPLO *vs* SIBs (p<0.01). The reason to re-admitted the patients in the hospital after tranplant was fever in 14 out of 28(50%) new admissions in UD setting ,11 out of 13(85%) in SIB and 7 out of 10 (70%) in HAPLO ; aGvHD was the cause for re-admission in 5 out of 28(18%) UD, 1 out of 13(8%) SIB and none in HAPLO. The other causes for re-admission were hemorragic cistitis, thoracic or abdominal pain. Second re-hospitalization is registered in 10 out of 61patients in UD (7 for aGHvD and 3 fever), 2 out of 54 (4%)in SIB (2 episodes of fever), 1 out of 36 (3%) patients in HAPLO (1 for fever and 1progressive disease). Also for second episodes, UD grafts had significantly more admissions compared to HAPLO and SIBS. Third re-admission was recorded only in UD patients (5 out of 61-8%). This study shows a comparable duration of admission for transplant for HAPLO and UD patients, both significantly longer than SIB grafts. The number of re-admissions is comparable in HAPLO *vs* SIBS and there is a trend for lower number of re-admission as compared to UDs.

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EFFICACY AND SAFETY OF BIOSIMILAR FILGRASTIM (ZARZIO®) AFTER AUTOLOGOUS STEM CELL TRANSPLANT: A PROSPECTIVE STUDY WITH HISTORICAL COMPARISON WITH LENOGRASTIM AND PEG-FILGRASTIM

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There is still skepticism about safety and efficacy of Zarzio® for prophylaxis of febrile neutropenia following conditioning chemotherapy and stem cell infusion, considering the lack of prospective studies with a long follow-up. From March 2013 to February 2017, 196 consecutive adult patients with hematologic malignancies (myeloma=110; lymphoma n=82; others n=4) underwent autologous stem cell transplant (ASCT) in our Institution. Zarzio® was given at the dosage of 5 mcg/Kg beginning to day 3 from infusion of stem cells and continued until neutrophilis recovery, with the aim to evaluate the efficacy and the safety of this biosimilar G-CSF. This cohort of patients was compared with two historical cohorts: a) 99 consecutive patients treated with Lenograstim (Myelostim®) at the same dosage given from day 3 after infusion from January 2009 to February 2013; b) 60 consecutive patients treated with peg-filgrastim (Neulasta®) 6 mg at day 3 after infusion from March 2006 to December 2008. The three patient cohorts were similar for all baseline features analyzed. The results of the study show a significantly shorter time to neutrophilis and platelet recovery (P=0.001 and P=0.007) in the cohort of patients treated with Neulasta®, whereas no difference was observed among the other two groups. We didn't observe any significant difference among the three patient cohorts for all the other analyzed parameters. In particular, we did observe a similar incidence of febrile neutropenia episodes (P=0.121), microbiologically documented infections (P=0.489) and needing of intravenous antibiotics (P=0.672). Moreover, we didn't find any significant differences as for transfusional needing (P=0.121), median hospitalization (P=0.194) and transplant-related mortality (P=0.856). No difference in terms of drug-related adverse events was observed in the three patient cohorts with no reported serious adverse events. Similar results were obtained performing two separate sub-analysis only for lymphoma or myeloma patients. Despite the limitations due to the non-randomized nature of the study, from our data on a large cohort of patients with a long-term follow-up biosimilar Filgrastim (Zarzio®) could be considered substantially equivalent in terms of efficacy and safety to Lenograstim (Myelostim®) and Peg-Filgrastim (Neulasta®), when used for hematological recovery and febrile neutropenia prophylaxis after ASCT in adult patients with hematologic malignancies.

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MONOCLONAL AND OLIGOCLONAL GAMMOPATHIES CONFER GOOD PROGNOSIS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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The occurrence of monoclonal and oligoclonal gammopathies (MGs) after allogeneic stem cell transplantation is a well-known phenomenon among physicians who follow-up transplanted patients, but the prevalence of this condition and its clinical significance remain still unknown. We retrospectively investigated the patients who underwent allogeneic stem cell transplantation at our Center from 1998, excluding all patients who presented monoclonal gammopathy before transplant. One-hundred ninety-four patients (57% male, 43% female) were included in the analysis. Sixty-three percent of them underwent transplant for acute leukemias (70% myeloid, 30% lymphoid), 11.8% for lymphomas, 10.8% for myelodisplastic syndromes, 5.1% for Ph-negative myeloproliferative neoplasms, 4.6% for chronic myeloid leukemias, 3.6% for other diseases. In 18 patients (9.3%) it was possible to report the presence of MGs (4 IgG kappa, 3 IgM lambda, 3 IgG lambda, 3 not characterized, 2 IgG lambda and oligoclonal, 1 IgG kappa and IgG lambda, 2 oligoclonal). There were not statistical association between sex, type of donor, conditioning regimen, source of stem cells, GvHD, CMV reactivation and appearance of MGs. The 2 patients transplanted for Chronic Myelo-monocytic leukemia both presented an IgG kappa MG, whereas all patients affected by chronic myeloproliferative neoplasrms did not develop MGs. The median time of follow-up was 9.4 months, whereas the median time of appearance of MGs was 6.4 months, with a median time of onset of IgM lambda MGs later than IgGs (28.9 *vs* 5.9 months). The Cox model showed a better trend in Overall Survival for patients with MGs (p= 0,06, HR 0,399). Five of 18 MG patients relapsed (27.7%), whereas in no-MG patients 69 experienced relapse (39.2%). Interestingly, 7 among 8 acute myeloid leukemia patients with MG were alive and in CR at the end of the follow-up time; all relapsed patients had IgG kappa MG. Patients with MGs showed a best PFS trend (p= 0.089, HR 1,7). None of the patients with MGs developed a plasmacellular dyscrasia in course of follow-up. In conclusion, the incidence of MGs after allogeneic stem cell transplantation is about 10%, but it is probably underestimated, and it could have a positive prognostic significance. Our results need confirmation in larger prospective data collections. However, the underlying immunological process could be of interest for its possible implication in graft-*versus* tumor effect and in other monoclonal gammopathies of unknown significance.

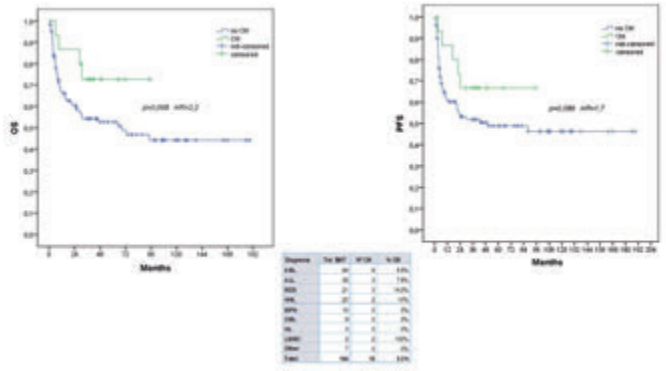

Figure 1.

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EFFICACY AND SAFETY PROFILE OF FOSCARNET AS PRE-EMPTIVE THERAPY FOR CYTOMEGALOVIRUS INFECTION IN HEMATOPOIETIC STEM CELL TRANSPANT RECIPIENTS

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Background: Cytomegalovirus (CMV) infection represents one of the most frequent infection after allogeneic hematopoietic stem cell transplantation (HSCT), and is treated pre-emptively either with ganciclovir or foscarnet. The two drugs have been compared in randomized trials and found to be equally effective. Renal toxicity is considered the limiting factor in patients receiving foscarnet. *Aims:* The aim of this study is to evaluate the efficacy and safety of foscarnet as pre-emptive treatment of CMV viremia, in a real-life setting in two established transplant Units, San Martino hospital in Genoa and Gemelli General Hospital Foundation in Rome. *Patients and Methods:* We retrospectively studied 106 patients (Table 1) who developed CMV viremia following allogeneic HSCT between 2010 and 2015. CMV infection was diagnosed either by antigenemia and/or PCR. Foscarnet was administered as first line (median dose of 155 mg/Kg) in 66 patients or as second or third line therapy (median dose of 97 mg/Kg), after failure of ganciclovir or valganciclovir in 40 patients. Ninenty-two patients received foscarnet alone and 14 patients received foscarnet combined with ganciclovir. The median duration of foscarnet treatment was 14 days (range, 2 to 91). Response was defined as clearance of viremia. Renal impairment was defined as an increased of creatinine level ≥100% from baseline and/or as a reduction of creatinine clearance ≥50% from baseline.

Hematological toxicity was defined according to NCI-CTCAE 4 criteria. *Results:* Median time of CMV antigenemia detection was 28 days (range, 0 to 1116) from HSCT. Overall response was achieved in 76% of cases. Unexpectedly, clearance of CMV was achieved more frequently in patients who received foscarnet as second or third line therapy as compared to first line (89% *vs* 69%, p=0.028). Fourteen patients received a combination of foscarnet and ganciclovir with a response rate of 100%. No patient developed CMV disease. Renal impairment was identified in 10% of patients, leukopenia in 17%, neutropenia in 29%, anemia in 8% and thrombocytopenia in 37%. None of these patients required foscarnet discontinuation. Overall survival was 70% and transplant-related mortality was 18%. *Conclusions:* In a real-life setting, foscarnet represents an effective pre-emptive therapy for CMV viremia after HSCT, with an acceptable toxicity profile, especially in cytopenic patients. Renal toxicity can be easily managed with dose adjustment and hydration.

Table 1. Patient's characteristics.

Patients	106
Median age years (range)	49 (17-74)
Recipient sex M/F	64/42
Disease	5 AA 6 HL 8 LNH 42 AML 14 MFI 15 ALL 1 MPD 3 CLL 3 CML 1 MM 8 MDS
Disease status	68 CR 1 PR 31 RR 6 SD
Conditioning MAC/RIC	60/46
Donor	9 REL 67 Haplo 28 MUD 2 MMUD
Stem cell source	80 BM 23 PB 3 CB
GvHD prophylaxis	1 MTX 6 CSA+MFA 1 CSA 1 CSA+Alemtuzumab 31 CSA+MTX 66 CSA+MFA+Cy
ATG	58/106
CMV serostatus D/R	48 +/+ 40 -/+ 6 +/- 4 -/- 8 missing

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BIOSIMILAR G-CSFS *VERSUS* ORIGINATOR G-CSFS FOR CD34+ CELLS MOBILIZATION AND AUTOGRAFTING

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Biosimilar G-CSFs (BioG-CSFs) were recently introduced in clinical practice to mobilize CD34+ cells and to reduce chemotherapy-induced neutropenia. This study was designed to evaluate their safety and efficacy in the "real life" setting. Patients treated with BioG-CSFs were compared with a historical cohort treated with originator G-CSFs (filgrastim or lenograstim). Primary endpoints were yields of CD34+ cell harvests and engraftment kinetics after autografting. Secondary objectives included transfusion requirements, duration of hospitalization, incidence of febrile neutropenia and of documented infections. Leukapheresis were performed for levels of circulating CD34+ cells >20/uL. Day of neutrophil and platelet engraftments were defined as the 1st of 3 consecutive days of absolute neutrophil count (ANC) >= 500/ul and the 1st of 7 consecutive days without transfusion support respectively. Overall, 266 patients, mainly with myeloma or lymphoma, were enrolled for a total of 343 autologous transplants. Seventy-seven myeloma patients received 2 planned autografts. Respectively, 138 and 127 patients received chemotherapy and originator or BioG-CSFs (5-10 ug/kg/day) to collect CD34+ cells. Less than 4% were poor mobilizers in both cohorts. No differences between cohorts were observed in time from chemotherapy to 1st leukapheresis (median day 11 *vs* 11 p=0.472) and circulating levels of CD34+/ul (mean 157/ul *vs* 177/ul, p=0.323); and overall CD34+ x 106/kg recipient harvested (mean 17 x 106/kg *vs* 15 x 106/kg p=0.209). After the autograft, day +25 cumulative incidences of neuthrophil engraftment were 99% *vs* 98% (p=0.714) in the originator (n=137) and BioG-CSFs (n=206) cohort. Day +25 cumulative incidences of thrombocytopenia differed, 98% in the originator *vs* 95% in the BioG-CSFs group (p<0.001). However no impact on clinical outcomes, including incidence of bleeding episodes, and transfusion requirements were observed. Duration of hospital stay, febrile neutropenia and incidence of documented infections were also comparable between cohorts. Serial measurements of complete blood counts were also investigated after discharge up to day +90. No differences in hemoglobin levels, platelets and ANC were observed at any time-point. In our experience, BioG-CSFs were as safe and effective as originator G-CSFs. Finally, the extensive use of BioG-CSFs led to a significant cost containment.

P241

ROME TRANPLANT NETWORK POLICY FOR ADULT PATIENTS WITH HIGH RISK ACUTE LYMPHOBLASTIC LEUKEMIA

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Between October 2006 and October 2016, 110 adult patients with high-risk ALL, all considered eligible for allogeneic transplant (ASCT), were referred to the Rome Transplant Network (RTN), a JACIE accredited metropolitan transplant program. Based on a pre-defined RTN policy, search for a donor followed a hierarchal selection: 1) HLA identical sibling (Id-sib); 2) matched unrelated donor (MUD); 3) Umbilical cord blood (UCB); 4) Haploidentical donor (haplo). All patients received the same myeloablative (n=60) or reduced intensity (n=10) conditioning regimen consisting of thiotepa, single daily dose of i.v. busulphan and fludarabine (TBF), including ATG in MUD, haplo and UCB transplant. As GVHD prophylaxis, CSA/MTX combination was given in all patients but UCB recipients whereas in haplo recipients MMF and an anti-CD-25 MoAb was added; in UCB transplant, CSA and PDN association was also given. Of 110 patients, 31 were not transplanted because of disease progression and death (n=21, 68%), donor unavailability (n=4,

13%) or loss of eligibility due to clinical reasons (n=6, 19%). Finally, 79 patients (72%) underwent ASCT: 30 from MUD, 24 Id-sib, 16 haplo and 9 UCB. Excluding UCB recipients due to the few numbers, 70 patients, with a median age of 35.5 years (18-65), were included in this preliminary analysis. According to the disease status at the time of ASCT, 31 patients were in first CR and 39 in > 2 CR or with active disease. With a median of 17 days, all patients had a full donor chimerism engraftment either for PMN (range,10-35) or PLTS (range,10-60). The median number of infused CD34+ was 4.6x10(E6)/kg (range, 0.6-12.4) with a significant correlation between cell dose and median time to PMN (p=0.003) and PLTS (p=0.0002) recovery. Overall, TRM at 100 days and 2-yrs was 11.4% and 28.5%, respectively; the relapse rate was 33% and the 5-yrs OS and LFS was 36% for both. The 5-years OS was 53% for MUD, 25% for Id-sib and 18% for haplo with a significant advantage of MUD compared to haplo-ASCT (p=0.05). Finally, patients transplanted in first CR experienced a significantly longer 5-yrs OS compared to the others (42% *vs* 18%, p=0.04). In conclusion, although disease progression occurring during donor search is the main obstacle (68%) to get access to ASCT, RTN policy allows a high proportion of eligible patients with high-risk ALL to be transplanted with some specific categories benefiting the most in terms of outcome.

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AN INCREASED FERRITIN SERUM LEVEL IMPAIRS PERIPHERAL BLOOD STEM CELL COLLECTION AND OUTCOME OF MULTIPLE MYELOMA PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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Iron overload represents an adverse prognostic factor for patients with hematological malignancies undergoing allogeneic stem cell transplantation (SCT). Recent observations have pointed out that pre-transplant ferritin serum level possesses a prognostic value even after autologous stem cell transplantation (ASCT) in multiple myeloma (MM) patients. Aim of the present study was to evaluate whether ferritin serum level, as detected prior to the chemotherapy regimen performed in order to promote peripheral blood stem cell (PBSC) mobilization, has an influence on PBSC collection procedure and transplant outcome. The records of all the patients who underwent autologous PBSC transplantation for MM at our Institution were reviewed; a pre-PBSC collection serum ferritin was available in 58 transfusion -naive patients (34M, 23F, median age=65yrs). PBSC mobilization regimens included Cyclophosphamide 2-4g/sqm+G-CSF in 55 patients and G-CSF+plerixafor in 3 patients. A ferritin serum level above normal (>300ug/L) was observed in 31 patients (53%). These patients did not differ significantly from those with a normal ferritin serum level in terms of disease status at PBSC mobilization, (CR 6% *vs* 11%); however a lower number of CD34+ cells x10^8/kg (8.7±3.1 *vs* 11.5±6.2, p=0.04) was collected, in a higher number of apheretic procedures (p=0.03). Post transplant response was similar in the two groups of patients, however both time to progression (21 *vs* 38 months, p 0.05) and overall survival (22 *vs* 49 months, p 0.04) were shorter in patients showing a higher ferritin serum level. CD34 cell collection and survival were not influenced by other acute-phase proteins such as CRP and fibrinogen. Our results suggest a possible influence of iron overload, or other putative iron- related mechanisms over outcome of MM patients undergoing ASCT.

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COMPARATIVE STUDY ON ATG-THYMOGLOBULIN VERSUS ATG-FRESENIUS FOR THE GRAFT VERSUS HOST DISEASE (GVHD) PROPHYLAXIS IN ALLOGENEIC STEM CELL TRANSPLANTATION FROM MATCHED UNRELATED DONOR IN RECENT YEARS: A SINGLE CENTRE EXPERIENCE

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Two different rabbit ATG formulations, thymoglobuline-ATG (tATG) and fresenius-ATG (fATG) are usually employed for graft *versus* host disease prophylaxis in allogeneic stem cell transplantation (allo-SCT). Herein we evaluated the differential activity of these two formulations in a cohort of 76 patients submitted to allo-SCT from matched unrelated donor in a single hematology center for hematological malignancies. Overall, 46 (60%) patients received fATG and 30 tATG as GVHD prophylaxis, in association to cyclosporine and methotrexate. The median follow-up of the entire cohort was 22 months (range, 1-88). fATG treatment was associated with a reduction of moderate-severe GVHD compared to tATG (HR 0.2, CI95%:0.04-1, p=0.05), with a cumulative incidence of 5,2% *versus* 27,6% at 2 years (p=0.03) in fATG and tATG group, respectively. No differences were observed between the two subgroups in term of incidence of acute GVHD, infection, transplant-related mortality, disease-free survival, and overall survival. However, a trend for a higher incidence of early-CMV reactivation was seen in the tATG cohort. A subset of 48 patients underwent immune recovery study by flow-cytometry analysis. As compared to tATG, fATG patients showed a significant lower percentage of CD3-CD56+ and CD3-CD16+ NK (15.5±2.3% *vs* 37.8±7.9%, p=0.001 and 14.6±2.3% *vs* 25.9±5.4% p=0.029 respectively), and lower CD3+CD4+CD25+ CD127low/- T-reg cells (0.68±0.1% *vs* 0.83±0.1%, p=0.013); on the contrary fATG-exposed patients presented a significant higher percentage of CD8+ T cells (55.3±3.1% *vs* 36.6±5.3%, p=0.002) and in particular CD8+ naïve T cells (25.3±2% *vs* 17.1±4.2%, p=0.05), with no differences in CD8+ memory cells at 3 months. In conclusion, this retrospective study shows that fATG-treated patients have a lower incidence of severe cGVHD compared to tATG. On the contrary, a slight increase of early CMV reactivation has been observed in tATG group. These differences do not result in a higher relapse-related or overall mortality. The distinct immune reconstitution as identified by flow-cytometric analysis, could justify these observations, that need to be validated by prospective studies.

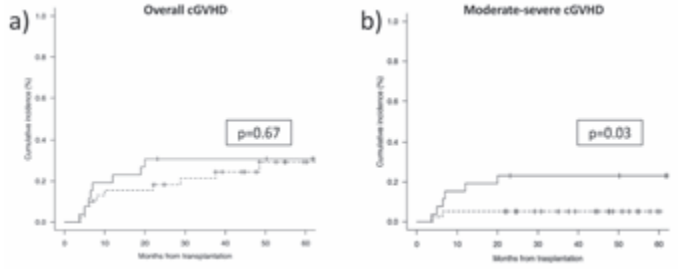

Figure 1.

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FEASIBILITY AND OUTCOME OF AUTOLOGOUS STEM CELL TRANSPLANT (ASCT) IN PATIENTS >70YRS: A SINGLE CENTER EXPERIENCE

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ASCT is considered the standard of care for young multiple myeloma (MM) and relapsed chemosensitive aggressive non Hodgkin lymphoma (NHL). This issue has recently become controversial in elderly patients, as even though the procedure is nowadays safer due to an improvement in supportive care, on the other hand, the availability of newer drugs could reduce the need to consolidate a response with high dose therapy. In this study we retrospectively analyzed the outcome of 34 consecutive patients (22 MM and 12 NHL) aged >70 (median age 71 yrs, range 70-76) who underwent a transplant program in our Institution from January 2007 to March 2017. PBSC mobilization was performed with cyclophosphamide 2-3g/sqm+G-CSF in all MM patients and with Ara-C or a polychemotherapy regimen (DHAP, IGEV) +G-CSF in lymphoma patients. No mobilization failures occurred and a median of 7.2 CD34+ cells x 10^8/kg was collected. Preparative regimen to transplant consisted of Melphalan 140 or 100 (7 patients) mg/sqm in MM patients and

BEAM or FEAM (3 patients) in NHL. Complete hematological recovery was achieved in all the patients (median=11 days for PMN >500/mmc and 12 days for platelets >20000/mmc) The whole procedure was well tolerated and no patients died within 100 days after transplant. Median disease -free survival was 20 months (23 months for MM patients) and median overall survival was 27 months (28 for MM patients). Our data suggest that ASCT can be safely performed in fit patients aged >70 yrs, thus representing a suitable therapeutic option even in the era of new drugs.

P245

THE TIMING OF PLERIXAFOR ADMINISTRATION MODULATES THE GRAFT COMPOSITION OF MM PATIENTS UNDERGOING STEM CELL MOBILIZATION WITH CY AND G-CSF: BIOLOGICAL AND CLINICAL RESULTS OF A PHASE IV MULTICENTER STUDY

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Plerixafor (AMD3100) is a selective, reversible inhibitor of CXCR4 inducing mobilization of haematopoietic progenitor cells from the bone marrow to the blood. It is approved for multiple myeloma (MM) and lymphoma patients poor mobilizers after G-CSF ± chemotherapy. However, the role of Plerixafor in modulating the kinetic of hematopoietic progenitor cell subpopulations and lymphocyte subsets in MM patients undergoing chemotherapy plus G-CSF front line has been poorly investigated. Aims of this study were: the percentage of patients collecting >6x10e6 CD34+ cells/Kg in <3 apheresis and the number of apheresis to collect >6x10e6 CD34+ cells/Kg; the cellular graft content; the engrafment and immunological reconstitution after transplantation of plerixafor-mobilized PBSC. In this prospective multicenter Phase IV study, we enrolled 37 patients with MM who would benefit from one or tandem ASCT. They received cyclophosphamide 4g/m^2 plus G-CSF 10mcg/Kg/die starting on day +6. Plerixafor (240 mcg/Kg/day) was added if WBC count were >1.0x109/L and platelets count was >20 x 109/L) regardless of CD34+ cell counts; up to 5 injections were allowed until the collection of >6x10e6 CD34+ cells/Kg. We evaluated the number of total CD34+ cells and the percentage of CD34+/CD38-, CD3+, CD4+, CD8+, CD19+, CD56+/CD3-, CD4+/CD25+/FOXP3+, CD138+/CD38+ cells on each apheresis. Standard ASCT was performed within two months from the last apheresis. Haematological and immunological recovery was determined at 30 days, 3, 6, 9, and 12 months after transplantation. Overall, 34/37 patients did mobilize PBSC and 30/34 patients collected >6x10e6 CD34+ cells/Kg (median number= 10.4x10e6/Kg in a median of 2 apheresis). Patients with lower CD34+ cells (<10/mmc) when Plerixafor was started (9 patients) had a higher increase of circulating CD34+ cells after plerixafor (range 6.27 to 63.36, median fold change 10.92). On the other hand, patients with higher CD34+ cells count (>20/mmc) had a reduced fold-change of circulating CD34+ cells after plerixafor administration, but showed a significantly higher percentage of CD3+ T cells in the graft, which correlated with more rapid immunological reconstitution at 9 and 12 months after transplantation. The data suggest that the timing of plerixafor administration in "normal mobilizers" MM patients, alongside its effect on PBSC mobilization, may also impact the graft CD3+ composition resulting in different immunological recovery after transplantation.

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LONG-TERM FOLLOW-UP OF PATIENTS UNDERGOING ALLOGENEIC TRANSPLANT FOR THERAPY-RELATED ACUTE MYELOID LEUKEMIA. EXPERIENCE OF THE ROME TRANSPLANT NETWORK

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From 2004 to 2016, 20 patients (pts: 8 males, 12 females, median age 51 years, range 24-63) with therapy-related acute myeloid leukemia (t-AML) underwent alloHSCT. One pt received chemotherapy for rheumatoid arthritis and 19 pts for a primary malignancy (PM): breast cancer (7), NHL (3), HL (2), breast cancer plus gastric NHL (1), testicular cancer (1), thyroid cancer (1), CLL (1), osteogenic sarcoma (1), lung cancer (1), AML (1). The risk by cytogenetic analysis for 18 pts was favorable in 3, intermediate in 7 and high in 8. All pts but 1 were included in this study. At HSCT, 15 pts were in 1 CR, 2 in 2 CR, 2 with refractory disease. Eight pts were allografted from HLA id sibling, 6 from haplo donor, 4 from MUD and 1 from double umbilical cord blood (UCB) units. The conditioning regimen was myeloablative in 11 pts and at reduced intensity in 8. As GVHD prophylaxis, the UCB recipient was given the association of prednisone, Cyclosporin and thymoglobulin, while all other pts received the association of MTX/Cyclosporin combined with thymoglobulin in HSCT from unrelated donor and with basiliximab and mycophenolate mofetil in HSCT from haplo donor. All pts engrafted: median time to ANC>500/uL and PLT >20.000/uL was 18 days and 22 days, respectively. Acute GVHD was of 0-I grade in 2 pts and II-IV grade in 4, chronic GVHD was extensive in 3 pts and limited in 1. At a median time of 7 months (range 3-26) from transplant 8 of 19 (42%) pts died. The causes of death were relapsed/refractory t-AML (n=5), acute GVHD (n=1), chronic GVHD (n=1), PM (n=1). Recurrence of previous breast cancer was observed in 2 of 7 pts (1 UCB, 1 haplo) at 18 and 24 months from HSCT, while in CR from t-AML. One died and 1 is alive in CR after chemo/surgery. Disease status at HSCT negatively influenced OS which was 58% at 26 months for pts transplanted in CR and 0% at 5 months for those not in CR (p<0.0001). With a median follow up of 62 months (range 3-129), 11 pts are alive in CR with an identical actuarial OS and DFS of 51% at 26 months. Athough on a limited number of pts, our results seem to confirm on a long follow up the curative potential of HSCT for pts with t-AML.

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COST-EFFECTIVENESS AND BUDGET IMPACT ANALYSIS OF LENOGRASTIM VS FILGRASTIM FOR MOBILIZATION OF PERIPHERAL BLOOD PROGENITOR CELLS IN PATIENTS WITH LYMPHOMA AND MYELOMA RECEIVING CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION

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The current economic context led the National Health Services to implement cost-effectiveness criteria to sustain decision-making processes of health technology use. Lenograstim (Leno), Filgrastim (Fil) and its biosimilar are Granulocyte-Colony Stimulating Factors (G-CSF) used for clinical transplantation to induce hematopoietic stem cells (HSCs) mobilization. A budget impact analysis and an incremental cost-effectiveness study of Leno use *vs* Fil have been performed. 248 pts undergoing autologous HSCs mobilization and HSCT have been evaluated and divided into 3 groups according to G-CSF used for HSCs mobilization and hematopoietic cells recovery after transplant: 100 were treated with Leno either for mobilization or hematological recovery, 93 initially treated with Leno and then with biosimilar Fil (Leno-Fil), 55 treated with biosimilar G-CSF (Fil) either for mobilization or post transplantation support (Fil-Fil). The efficacy of post-transplant recovery was evaluated considering number of G-CSF vials used, the required days to recover an adequate level of WBCs and PLTs, hospitalization days, percentage of patients who experienced adverse events (AEs). The analysis of the 3 cohorts of pts showed statistically

significant differences: a higher number of harvested CD34+ cells in Leno group *vs* Fil group. No statistically significant differences were found regarding the number of G-CSF vials, apheresis number and CD34+ cells peak. The post transplant hematological recovery was faster in Leno group compared with Fil group: median neutrophils >500/mmc 12 *vs* 13 days, median platelets >20.000/mmc, 13 *vs* 15 days (p<0,0001). Regarding the AEs, in Leno group was observed a lower pneumonia and fever incidence and a higher sepsis incidence to Fil group. In terms of budget impact, Leno use would allow a lower funds absorption amounting to - euro566/patient. These lower costs are related to a fewer days of hospitalization (-822 euro), overall lower incidence of AEs (-182 euro), laboratory tests, transfusions for platelets recovery following discharge (-115 euro), that balance the higher cost associated with the G-CSF (Leno) used (+302 euro for apheresis and +251 euro for bone marrow recovery). In terms of cost-effectiveness, Leno use allows to obtain better results for all measured parameters. Therefore, Leno is dominant to Fil in terms of efficacy and lower costs. The study revealed a clinical superiority of Leno *vs* Fil postulating a potential cost savings favouring Leno over Fil.

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A RETROSPECTIVE STUDY ON AML PATIENTS SUBMITTED TO A MODIFIED POST-TRANSPLANT CYCLOPHOSPHAMIDE (PT-CY) REGIMEN, FOLLOWING UNMANIPULATED HAPLOIDENTICAL BONE MARROW TRANSPLANTATION.

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Haploidentical bone marrow transplantation (HAPLO-BMT) with post-transplant cyclophosphamide (PT-CY) is being increasingly used in patients with acute myeloid leukemia (AML) who lack a suitable HLA-matched donor. The standard Baltimore regimen calls for PT-CY 50 mg/kg on days +3 and +4, with a calcineurin inhibitor and mycophenolate (MMF) starting on day +5 after transplant. We have modified the original Baltimore regimen (BBMT 2013; 19:117), and are now reporting our analysis of HAPLO-BMT in 31 patients with AML. All patients received a uniform GvHD prophylaxis, consisting of cyclosporine (CsA) and mycophenolate (MMF) starting on day 0, and PT-CY 50mg/kg, on days +3 and +5.

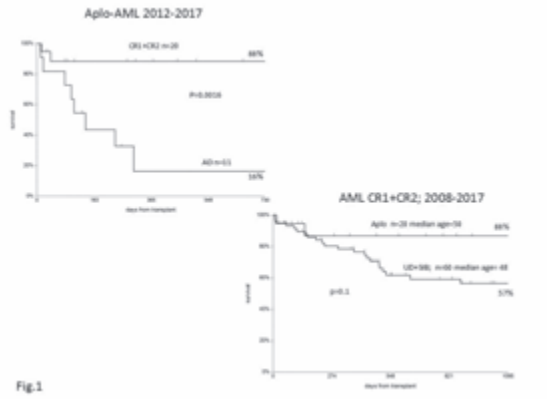

Figure 1.

All patients received umanipulated haploidentical marrow between year 2012 and 2016. Clinical characteristics included: 13 males and 18 females, median age of 50 years (18-72); low ELN risk group (6%) intermediate risk (42%) and high risk (52%); first complete remission (CR1) (39%), second CR (CR2) (26%) and active disease (35%). The median dose of TNC infused was 2.38x10^8/kg (range 1.2-5.2). All patients received a myeloablative regimen: either thiotepa (10mg/kg), busulfan (3.2mg/kgx3), fludarabine (50 mg/m^2x3) (TBF). Busulfan was reduced in patients over 60 years. The median follow up for surviving patients was 372 days (30-1740). Twenty-eight patients (90%) engrafted; the median interval to a neutrophil count of 0,5x10^9/L was day 19 (range 13-36). The 100 day cumulative incidence (CI) of grade II-IV and III-IV aGVHD was 13% and 6%. Chronic GVHD was observed in 5 patients with a cumulative incidence of moderate and severe cGVHD of 13% at 3 years. The cumulative incidence of transplant related mortality (TRM) at 5 years was 19%. Patients in CR1+CR2, or with active disease, have an actuarial 2 year overall survival (OS) of 88%, and 16%, respectively (p=0,0016). We favourably compared these results with data from our cohort of AML submitted to AlloSCT from UD or HLA sibling in the last 10 years. This study shows that our modified PT-CY regimen can be successfully applied in a setting of unmanipulated HAPLO-BMT for AML. For CR1,CR2 patients the outcome is excellent in terms of TRM, and survival, also in patients over 60. Relapse remains a problem in patients with active disease, as seen with any conventional transplant platform. The incidence of severe acute and chronic GvHD is very low, with marrow as the only stem cell source for all patients.

Quality of Life

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CHANGING TREND IN LOCAL BACTERIAL EPIDEMIOLOGY :EXPERIENCE OF BEHAVIOR/RESULT IN ACUTE LEUKEMIA PATIENTS HOSPITALIZED IN SINGLE HEMATOLOGY UNIT

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The intense chemotherapic regimens and hypometilant agents to treat acute leukemia induce prolonged neutropenia with high risk of infections.All 100 cases of Acute Leukemia (AL) admitted in our ward from august 2013 to February 2017 received prophylactic antibacterial therapy with fluoroquinolones and were analized for weekly routine tissue colture screening and serial blood colture for fever. Six patients were Lymphoid AL and 94 were Myeloid AL. 41 patients were not elegible for intensive chemotherapy(for age and comorbidities) and were treated with hypometilant agents, while 59 were younger than 65 years and were treated with induction /consolidation chemotherapy 3 plus 7 regimen.Median age was 58 years with range from 27 to 88 years old. We found 28 patients (28%) bacterial septic shock during fever, of which 20 cases gram negative (71%) in particular 65% E.Coli, 15% Enterobacter, 10% Klembsiella, 5% Stenotrophomonas, 5% Pseudomonas; while 8 patients (29%) had a gram positive septic shock (S. Haemoliticus 38%, S.capitis 25%, S. hominis 25%, S epidermidis 12%). During intensive chemotherapy and prolonged severe neutropenia we took over the major incidence of septic shock (23 patients 82%) than hypometilant treatment in particular decitabine (5 patients18%). During 2014 we had 3 mortal septic shock for multiresistant gram-klembsiella and Pseudomonas. Since than we adopted in our ward, isolation of patients with gram negative (klembsiella or pseudomonas)tissue colture positive, hygenic and sanitary practices with closing room for 48 hours and hand disinfection before entering and after leaving any patients room.We noticed a change of bacterial infections incidence in these 3 years in our ward.:reduction klembsiella /pseudomonas multiresistant infections and emergency of E.coli and Staphilococcus septic shock not multiresistant. More epidemiological analysis in several haematological ward are necessary to understand if is changing local microbial epidemiology or is the different management of neutropenic patients with acute leukemia with a different antimicrobial strategy to determine a changing trend.

P250

SUBCUTANEOUS BORTEZOMIB DELIVERED AT HOME FOR PATIENTS WITH MULTIPLE MYELOMA: A SUSTAINABLE MODEL OF DOMICILIARY CHEMOTHERAPY FOR HEMATOLOGY PATIENTS. A REPORT FROM REGGIO EMILIA HEMATOLOGY UNIT

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In order to reduce discomfort and mantain quality of life among unfit and frail hematology patients with advanced age, poor performance status, severe immunodeficiency and/or significant comorbidities, home care is a valid integration to the standard in-hospital hematology services. Patients affected by multiple myeloma (MM) presenting vertebral lesions, severe bone pain and walking limitations are the ideal candidates for a domiciliary program of supportive and palliative care in all steps of disease, from the onset to the terminal phase. Despite the increasing availability of oral anti-myeloma agents, bortezomib is a common therapeutic option, even in elderly patients and in the early phases of disease, but subcutaneous route of administration may represent a logistic restraint for those patients having serious difficulties to be transported to the hospital. In our Hematology Unit, from November 2014 to April 2017, 7 MM patients referred to the home care service have undergone subcutaneous bortezomib delivered at home. At the start of domiciliary treatment median age was 74.8 years (range 71-80), median ECOG performance status score was 3.3; six patients were at stage III and one at stage II according to the ISS prognostic system. Bortezomib was combined with melphalan and prednisone in 3 cases (VMP at first line), with thalidomide and dexamethasone in one patient (VTD at first line) and, in the remaining 3 patients, with dexamethasone alone (VD at third/fourth/fifth line). The total number of injections was 127 (range per patient: 2-31). On average every home access required 35 minutes (including a median trip of 10 km by car). Because of remarkable clinical improvement three patients were referred back to the day-hospital before or soon after the end of treatment. Two patients interrupted the treatment due to lack of response and progressive disease. Response rates for the six patients who completed or suspended the treatment were: CR=1, VGPR=2, PR=1, SD=1, PD=1. No serious adverse event was reported as a direct consequence of injections of bortezomib. Awaiting further evaluation on cost-effectiveness analysis and other unexplored issues, this innovative procedure of domiciliary chemotherapy, entrusted to a fully dedicated home care team (nurse and hematologist both with expertise in palliative care) partly funded by a no-profit association, seems very promising in terms of sustainability, safety and patient and family satisfaction.

P251

HOME CARE MANAGEMENT OF PATIENTS WITH ACUTE LEUKEMIA FROM ACTIVE THERAPY TO END-OF-LIFE AND PALLIATIVE CARE: A THREE-YEAR EXPERIENCE OF A SINGLE CENTRE

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Acute leukaemias are more frequent in elderly patients (aged 60 or older) and carry a very poor prognosis. Quality of life should be considered as one of the most important therapeutic targets and physicians should keep in great consideration the patients' perspective on time spent in the hospital and the medical care received throughout their illness and at the end of life. Patients with acute leukaemia face significant challenges at home and in hospice and there is a significant need to improve the end of life treatment and supportive care interventions in this setting. Home care management of patients with acute leukaemia by specialized health personnel, whenever possible, allows patients and their caregiver to reduce time spent inside the hospital and helps them to maintain a lifestyle as normal as possible. We conducted a retrospective study to describe the 3-year experience of our Haematological Home Care program focusing particularly on home care management of acute leukaemia patients in different stages of disease. We included in the study 44 patients with acute leukaemia.

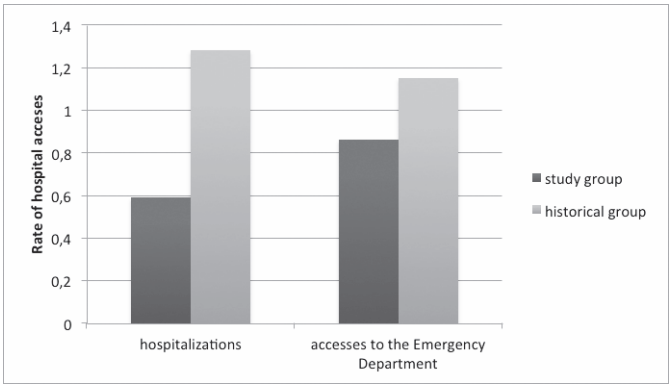

Figure 1. Rate of hospitalization and accesses to the Emergency Department by study group Among the 44 patients in the study group the total number of hospitalizations was 26 (0.59 per patient, range 0 to 4) and the number of accesses to the ED was 38 (0.86 per patients, range 0 to 7). Among the 7 patients of the historical group the total number of hospitalizations was 9 (1.28 per patient, range 0 to 3) and the number of accesses to the ED was 8 (1.15 per patient, range 0 to 3). The difference was not statistically significant (P=0.187 and P=0.472 for hospitalization and ED access, respectively).

The majority of these patients (36/44, 82%) needed a palliative treatment, while only 8/44 (18%) had an ongoing active therapy. We com-

pared data from our cohort of patients with those from patients with acute leukaemia assisted at home in the previous 3 years, when the HC program was still not active. In the study group the total number of hospitalizations was 26 among 44 patients (0.59 per patient *vs* 1.28 per patient in the control group) and the number of Emergency Department visits was 38 among 44 patients (0.86 per patients *vs* 1.15 per patient in the control group). Place of death was home environment in 53% of cases (*vs* 17% in the control group), hospice in 8% of cases (*vs* 17% in the control group) and hospital in 39% of patients (*vs* 66% in the control group). Home care for patients with acute leukaemia is feasible both for cases with potentially curable disease and for those with advanced disease and a short life expectancy. In particular this study suggests that in this patient population home care reduces time spent in hospital, decreases the number of hospitalizations and has positive effects on quality of life.

P252

PROMOTION OF QUALITY OF LIFE EVALUATION DURING EARLY PHASE OF HEMOPOIETIC STEM CELL TRANSPLANTATION: THE EXPERIENCE OF THE ROME TRANSPLANT NETWORK

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Introduction: At the beginning of 2016, the Rome Transplant Network (RTN) Quality of Life (QoL) Working Party (WP) has started a project for the dissemination of QoL assessment in the clinical practice during transplant and the development of a QoL report for clinical purpose. *Methods:* The EORTC questionnaire (QLQ C30) was chosen and starting on January 2016 transplant centers were encouraged to distribute the questionnaires to patients, at 3 time points: admission (t0), during aplasia (ta) and discharge (t1); the completed questionnaires were sent to the RTN QoL WP evaluation unit, for questionnaire processing, implementation of the report and mailing the report to the respective transplant center. The processing unit calculated the scores as reported in the questionnaire instruction manual and then reported the results in graphical form on the report, where functional scales and symptom scales scores were both numerically reported and evidenced with conditional formatting, that uses a color scale to differentiate scores. The report also included an explanation / suggestion box, where both the results of the QoL questionnaire were summarized in words and tips to improve QoL or prevent QoL impairment were suggested. Recommended or suggested procedures were performed as icons evidenced on the report; icons were established for: rehabilitation procedures (assisted or unassisted); psychological care, pain management, prevention of nausea and vomiting and nutritional support. *Results:* From January 2016 to May 2016 (4 months) 95 questionnaires were sent to the evaluation unit. Four out of 6 centers sent questionnaires. Overall, out of 80 transplants performed, questionnaires were administered and sent in 43 transplants (53.8%); the number of QoL assessments was 1, 2, 3 and 4 in 8 (19%), 19 (44%), 15 (35%) and 1 (2%) transplant, respectively. Out of 95 questionnaires, t0, ta and t1 assessments were 42 (44%), 25 (26.5%) and 28 (29.5%), respectively. Time interval between the date of questionnaire completion and the date on which the report was mailed was 2 days (median, range 0-40). *Conclusions:* The administration and processing of QoL questionnaires, and the reports implementation is feasibile, although adherence should be enhanced. Key points are: 1. the creation of a dedicated evaluation unit; 2. the development of an explanatory report to simplify QoL comprehention; 3. the suggestion of supportive care measures driven by patient-reported outcomes.

P253

PREVALENCE OF METABOLIC SYNDROME AND SARCOPENIA AS LONG-TERM LATE EFFECTS IN LYMPHOMA SURVIVORS

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Background: Metabolic syndrome and sarcopenia could often occur in long-term cancer survivors. The first is characterized by a set of cardiovascular risk factors defined by the Cholesterol Education Program Adult Treatment Panel III, which requires simultaneous presence of at least 3, abdominal obesity, hypertension, hyperglycemia, hypertriglyceridemia and low to high density lipoprotein cholesterol (HDL-C), sarcopenia is characterized by a progressive lean mass loss that causes asthenia and malnutrition. The aim of this study was to evaluate the prevalence of metabolic syndrome and sarcopenia in lymphoma survivors and provide adequate nutritional support according to Mediterranean Diet. *Patients and Methods:* Since November 2016 to March 2017, we enrolled 41 consecutive patients (19 women and 22 men) aged between 24 and 76 years in continuous remission of lymphoma for at least 3 years and in current follow-up at our Institution within the "CCM2014 project supported by the Italian Ministry of Health. Nutritional status was assessment by anthropometry (arm, wrist, waist, thigh and calf circumference), plicometry (according to Durnin Womerslay) and body mass index, while glucose, HDL-cholesterol, triglycerides were tested by immunometric assay; For each patient, a customized food plan has been developed based the Mediterranean Diet and they were followed every four weeks. *Results:* 16/41 (39.0%) of patients of both gender presented a status of obesity (mild, moderate and severe),12/41 (29,2%) were overweight, 11/41 (26,8%) were normal weight and 2/41(4.8%) were underweight; In the women the waist circumference mean was 60.4 cm (range:70-116), while for men the mean was 90.27 cm (range: 69-142). Considering the parameters that characterize the metabolic syndrome, 15/41 patients (36,5%) had at least 3 of these, significantly associated with status of obesity or overweight ($p<0.001$); Regarding the evaluation of the compartments by plicometry, a significant loss of lean mass and consequent increase in fat mass was and malnutrition observed in the obese and overweight patients respect to normal weight ($p <0.001$). *Conclusions:* More than 60% of long-term lymphoma survivors have a moderate or severe weight gain and 36% have metabolic syndrome associated to sarcopenia; these preliminary data suggest that an early nutritional intervention associated with adequate physical activity could reduce the risk of onset of both complications in lymphoma survivors.

P254

A POTENTIAL ROLE OF GONADOTROPIN RELEASING HORMONE AGONIST TO PREVENT CHEMOTHERAPY-INDUCED OVARIAN DAMAGE IN ACUTE LEUKEMIA PATIENTS

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Anticancer treatments have improved survival among patients with hematologic malignancies and long-term side effects of chemotherapy (CHT), like infertility have become a major issue. Embryo/oocyte cryopreservation are standard strategies for fertility preservation in women. In order to evaluate the impact of acute leukemia and its treatment on fertility and the efficacy of temporary ovarian suppression with gonadotropin-releasing hormone agonist (GnRHa) or Oral contraceptive pill (OCP) during CHT, we retrospectively analyzed a population of 39 premenopausal women of median age 37 years (range 20–49), treated for AML (66.7%, 26 patients) or ALL (33.3%; 13 patients), since 2005 to 2016. The efficacy of temporary ovarian suppression was evaluated by the return of spontaneous menstruation and rate of pregnancy after the end of CHT.37 of 39 patients received GnRHa (leuprorelin acetate monthly administration) alone or in association with OCP during CHT. Conversely, 1 patient died before starting administration and the other one had previously undergone hysterectomy. Among these 37 patients, only 23 could be evaluated in term of preserved ovarian function defined as regular menses whereas 11 (29.7%) early died and 3

(8.1%) were lost in follow up. 12 patients (52.2%) underwent HSCT, namely 3 autologous and 9 allogeneic. The median duration of GnRHa therapy was 10 months (range 3-43). 11 patients (47.8%) resumed menses within a median of 4 months after termination of treatment. Pregnancy occurred in 2 patients (8.7%).Current options for fertility preservation in cancer patients during CHT may not be feasible in acute leukemia because of the impossibility to delay CHT and the risk of recurrent disease after reimplantation of contaminated ovarian tissue. In our study, despite the limited patients' cohort and the absence of hormone levels monitoring, the use of GnRHa alone or with OCP is safe, effective in preventing heavy vaginal bleeding. The return of spontaneous menstruation seems to be more associated with treatment intensity: only one transplant patient resumed ovarian function after 40 months since CHT and subsequent estroprogestinic treatment. This is a critical issue because HSCT frequently represents the best treatment for adults with leukemia. Actually we propose the dosage of sex hormones to patients in fertile age with newly diagnosed hematologic malignancies. Other studies are necessary to investigate the role of GnRHa in fertility preservation.

P255

A NEW INTENSIFIED ORAL HYGIENE PROTOCOL CONFERS A BENEFIT ON GRADE OF MUCOSITIS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Oral mucositis is a common complication of allogeneic stem cell transplantation (allo-SCT). The damage due to radio-chemotherapy increases the risk of bacterial superinfection. Protocols of oral hygiene play an important role to prevent the onset of oral infections and may reduce the disconfort due to mucositis. We performed a perspective, monocentric clinical trial in which we used professional oral hygiene and specific dental aids. We enrolled 10 patients undegoing myeloablative allo-SCT for haematological malignancies at our institution. The protocol included a set of specific tools joined to standard procedures (toothbrush, clorexidine mouthwashes), with a daily schedule of application. An oral hygiene session was performed before the date of recovery. The protocol provided from the first day of hospitalization until stem cells infusion: manual toothbrush with bristles of 0,12 mm of diameter, super-soft; antibacterial toothpaste (Bioxtra®) containing colostrum and antimicrobial enzymes; antibacterial mouthwash (Oralys®) containing lisozyme, lactoferrin, lactoperoxidase and extracted colostrum, fluorine, xilitole and aloe vera. From day +1 after stem cell infusion until engraftment or resolution of mucositis: manual toothbrush with bristles of 0,10 mm of diameter, ultra-soft; mouthwash (Mucosyte®) containing maltodextrin, propylene glycol, hydroxyethylcellulose, sodium hyaluronate, sodium saccarhin and citric acid. Patients were clinically supervised every day by physicians and dental hygienists through clinical examination, WHO grading of mucositis and VAS scale for the pain. All patients were conditioned with TBF (Thiothepa, Busulfan, Fludarabine). The data collected were compared to a historical cohort of 10 transplanted patients who received only standard procedures. Patients enrolled in the oral hygiene protocol had a lower pain reported in VAS scale, a significant lower level of mucositis, and a later onset of mouth lesions, when compared to the historical group. Additionally, enrolled patients had a notably lower need of analgesic drugs (opioids). In conclusion, these findings demonstrate the importance of specific oral hygiene in preventing high-grade mucosytis after allo-SCT, suggesting the importance of dental and oral hygiene in the bone marrow transplant setting.

P256

USE OF COMBINED ORAL ADMINISTRATION OF ANALGESIA AND ANXIOLYSIS FOR PAIN ASSOCIATED WITH BONE MARROW ASPIRATION AND BIOPSY

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Bone marrow aspiration and biopsy (BMAB) is a painful procedure, and the local infiltration anesthesia (LIA) with lidocaine is unable to relieve the pain during the most uncomfortable phases, or the anticipatory anxiety related to pain recalling thereafter. Our randomized and patient blinded trial aimed to evaluate, as primary end point, the efficacy and safety of opioid and benzodiazepine agent combination plus LIA in patients who underwent BMAB for hematological malignancies. Two secondary end points were: 1) define if patients who already underwent to BMAB without LIA prefer sedoanalgesia; 2) sedoanalgesia can influence the quality of the biological specimen harvested. Patients were randomly assigned into two arms for receiving either sedoanalgesic placebo plus LIA (standard group, 48,6%) or oral fentanyl citrate 200 mcg plus oral midazolam 5 mg in addition to LIA (combo-group, 51,4%) during BMAB. Pre-procedural anxiety and procedural pain were assessed according to the Numbered Rating Scale (NRS: 0-10), dividing the time of the procedure into five intervals (T0, T1, T2a, T2b, and T3) and evaluating discomfort grade during each moment of procedure in both groups.

Table 1.

Table 1. Baseline characteristics of the population undergone to BMAB

Characteristic	No.
Number of patients	107
Sex	
Male/Female	9/5
Age, years	
Median (range)	61 (19-84)
Type of hematologic neoplasm	
Acute myeloid leukemia	19
Non Hodgkin lymphomas	16
Myelodisplastic syndromes	16
Multiple Myeloma	13
Essential thrombocythemia	11
Polycythemia vera	10
Chronic lymphatic leukemia	7
Hodgkin lymphoma	6
Primary myelofibrosis	5
Acute lymphoblastic leukemia	5
Chronic myeloid leukemia	4

Cognitive function was measured before and 30 minutes after the procedure. Possible side effects were recorded, as well as the adequacy of tissue samples harvested. A telephone interview was performed 24 hours later. A total number of 116 patients were enrolled in the study. Nine patients did not meet inclusion criteria and were excluded. Fifty-two patients were randomized and assigned to standard group and fifty-five to combo group. At T2b and T3 (biopsy time and time after the biopsy, respectively) there was a significantly lower ($p<0.05$) perception of pain in the patients who received sedo-analgesia (combo) compared to those who did not (standard). Moreover, 100% of the patients in combo group who had previously undergone this procedure without premedication, reported that they would prefer sedoanalgesia for the subsequent procedures. Finally, the histological specimen was found to be high in quality, as defined by standards. Administration of oral analgesia and anxiolysis is a safe and feasible option to be used in outpatient setting; sedo-analgesia is very effective in reducing pain during the biopsy and diminishes the anticipatory anxiety related to a painful procedure. Patients should have the possibility to choose between local anesthesia alone or sedo-analgesia plus local anesthesia.

P257

QUALITY OF LIFE IN HEMATOLOGICAL PATIENTS UNDER PROLONGED ANTICOAGULATION FOR HIGH-RISK DEEP VEIN THROMBOSIS: A LONG TERM FOLLOW-UP

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Le attuali linee guida indicano trattamento anticoagulante prolungato in pazienti oncologici con trombosi venosa profonda (TVP), la gestione a lungo termine del tromboembolismo venoso è tuttavia ancora controversa soprattutto in relazione alla compliance dei pazienti oncologici. Qui riportiamo i risultati di un follow-up a lungo termine in pazienti con TVP sottoposti a trattamento anticoagulante con Eparina a Basso peso Molecolare (EBPM) in relazione a presenza o meno di trombo venoso residuo(RVT) alla CUS, dopo sei mesi di terapia anticoagulante standard. I pazienti sono stati sottoposti a valutazione clinica e indagini di laboratorio ogni tre mesi o prima, se necessario.Tra i principali end

points dello studio vi è stata la valutazione di qualità di vita in pazienti sottoposti a trattamento quotidiano con iniezioni sotto-cute. La qualità di vita è stata valutata tramite questionario EORTC-C30, somministrato da una psciologa all'arruolamento e durante ciascuna visita di follow-up. Nell'arco di tre anni sono stati arruolati 230 pazienti (121 M,109 F, età media: 61.1aa) affetti da neoplasia attiva (69 neoplasie ematologiche) con riscontro CUS di RVT dopo un primo episodio di TVP.Al tempo dell'analisi, 146 soggetti erano vivi. Gli score medi globali risultati dall' EORTC-C30, all'arruolamento e dopo ventiquattro mesi, sono stati, rispettivamente pari a 52.1 e 50.1. Non sono state riscontrate differenze significative tra gli score all'arruolamento e dopo ultimo follow-up (p: 0.001). Il trattamento a lungo termine con EBPM è risultato ben tollerato e privo di impatto negativo sulla qualità della vita nei pazienti arruolati.

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PU001

COMPLETE BLASTOSIS REMISSION IN A PATIENT WITH RAEB-2 SECONDARY TO MDS/MPN TREATED WITH AZACITIDINE: A CASE REPORT

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Azacitidine (Aza) is a hypomethylating agent approved for the treatment of myelodysplastic syndromes with Intermediate-2 or High IPSS risk. We report a 72 years old male patient with a history of myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) treated with rHuEPO and hydroxyurea. Subsequently the patient developed a refractory anemia with excess blasts (RAEB-2) with a bone marrow blastosis of 15% and a High IPSS score risk. Patient presented severe anemia with transfusion-dependence (TD) of 4 RBC units/month, thrombocythosis, no splenomegaly. We started treatment with azacitidine (75 mg/mq/die d 1-7 every 28 days), hydroxyurea and deferasirox (20 mg/Kg/die). After 6 cycles TD decreased to 1 RBC unit/month and bone marrow blastosis decreased to 5%. Therapy with Aza was well tolerated so we decided to continue treatment. After 12 cycles TD was stable, bone marrow blastosis decreased still to <2% according with complete remission but the bone marrow showed a fibrosis grade 2 (previously not present). Treatment with Aza in accelerate phase of MDS/MPN can be useful to decrease blastosis despite progression of the myeloproliferative feature in myelofibrosis.

PU002

A PATIENT WITH MYELODYSPLASTIC SYNDROME WITH ISOLATED DEL(5Q) AND IRON OVERLOAD TREATED WITH LENALIDOMIDE AND DEFERASIROX: A CASE REPORT

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Lenalidomide (Lena) is approved for treatment of myelodysplastic syndrome (MDS) with isolated del(5q) and low/Intermediate-1 IPSS risk. Deferasirox (DFX) is an iron chelator approved for treatment of iron overload in myelodysplastic syndrome. We report a case of a 78 years old female patient, with diagnosis of MDS with isolated del(5q), low IPSS risk. The patient presented a severe anemia with transfusion-dependence of about 4 RBC units/month. The patient showed an iron overload (sieric ferritin level about 2500ng/ml) and slight thrombocytopenia. So we started Lena 10 mg/die d 1-21 every 28 days in combination with DFX 10mg/kg/die. We checked liver and kidney functionality every week for the first month and then every month. Both liver and kidney function test values were within normal limits. After 2 cycles of treatment with Lena, hemoglobin level increased to normal range (12-13 g/dl) but neutrophils granulocytes decreased to <1000/µl. So we decided to reduce Lena dose to 5mg/die d 1-21 every 28 days from the next cycle. After 12 cycles of treatment with Lena an additional decrease of neutrophils granulocytes <1000/µl was still reported so we decided to further reduction of Lena to 2,5mg/die d 1-21 every 28 days; ferritin level was 1000-1500ng/ml so we continued with DFX 10mg/Kg/die. After 18 cycles of treatment with Lena, hemoglobin level remains in normal range as well as neutrophils granulocytes, however thrombocytopenia remains still slight and ferritin level is about 1200ng/ml. Despite both Lena and DFX are nephrotoxic, the liver and kidney functionality remain in good clinical condition without signs of worsening. Treatment with Lena plus DFX in MDS with isolated del(5q) and iron overload is safety. Morover, in our experience, Lena 2,5mg/die d 1-21 every 28 days can be a good alternative for patients who develop neutropenia for higher dosage.

PU003

WILMS TUMOR 1 (WT1): COULD IT BE USED AS NEW PROGNOSTIC MARKER IN RAEB MYELODYSPLASTIC SYNDROME FOLLOW UP IN ALLOTRANSPLANT PATIENTS?

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The Myelodysplastic Syndromes (MDS) are a heterogeneous group of blood diseases in which bone marrow either release abnormal cells that undergo apoptosis, or originate damage cells that are released into the peripheral blood impaired functional capacity. Molecular alterations and complications of MDS are related in relation to the level of the cells differentiation stage involved. According to FAB classifications, suggest 5 type of MDS. Our aim is to seek a marker which can be able to facilitate the diagnostic classification, monitor the therapeutic treatment response over time and follow the minimal residual disease. The study was applied to a patient 65 years old, Caucasian, patient not exposed environmental non-toxic and without a work activity at risk. In patients with suspect diagnosis of MDS, we use DNA and RNA extracted from bone marrow samples. The case presented blasts to 16%, partial myeloperoxidase expression; the immune phenotype analysis show 16% of immature myeloid cells, Bone marrow stain show morphology dysplastic in the red series, in granulocytic and megakaryocytic cells. Cytogenetic evaluation at onset is complex and highlights: aberration of chromosomes 2 and 3, translocations of chromosomes 5and 7, translocations between chromosomes 11 and 12, rare aberrations of chromosome 6 are detected. We evaluated all molecular marker panel of acute myeloid leukemia in our patient who has 16% of blasts; all results are negative, except for quantitative PCR evaluation of WT1: 13570 copy /10000 abl. One month after diagnosis of Myelodysplasia (RAEB)the patient was subject to allogeneic transplantation from family donor, with 5.34 x 10E6 CD34/kg cells infusion, and monitored quantitatively by WT1 evaluation and cytogenetic analysis. The diagnostic path used for a correct diagnosis is often difficult due to absence of specific molecular markers in Myelodysplasia Syndrome. The quantification of WT1 over expression at diagnosis allowed to use the new marker in RAEB MDS and could it be used as a prognostic indicator before and after therapy transplantation. WT1 is found to be predictive, sensitive, compared to the study of chimerism; WT1 evaluation was confirmed by cytogenetic analysis results. The multidisciplinary diagnostic approach and WT1 evaluation as new marker has proved successful in studying and monitoring of Myelodysplasia RAEB Syndrome.

PU004

DEFERASIROX AND ISOLATED JAUNDICE: A NEW SIDE EFFECT OF ORAL IRON CHELATION THERAPY FOR DELTA-BETA THALASSEMIA

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Since several years, Deferasirox (Exjade®Novartis), has been used for oral iron chelation therapy of pediatric patients older than 6 years affected with transfusion-dependent hemoglobinophaties and poor response or compliance to Deferiprone therapy. Many side effects have already been described, such as renal and tubular dysfunction, cytopenia, liver dysfunction, and others. We are going to describe the case of a 13 years old patient with delta-beta Thalassemia, who presented isolated jaundice as new side effect probably related to the switch therapy from Deferiprone to Deferasirox. At the beginning of the therapy with Exjade, the child was in good clinical condition. He underwent a surgical splenectomy and colecystectomy at the age of 11. Associated comorbidity was VII Factor defect. He required blood transfusion with a median interval of 21 days (range 15-28 days). Ferritin serum levels were over 1159 ng/ml, although Deferiprone therapy 6 days/week and the absence of iron overload signs at RMN T4 STAR. Therefore Deferasirox therapy was started with the usual dosage of 20 mg/kg/day. After two months from starting therapy the patient showed an isolated jaundice, with mixed bilirubin increased values (max values: total and

indirect bilirubin 6,57 and 6.19 mg/dl, respectively). Liver dysfunction, signs or symptoms of hemolysis, renal or other organs failure and viral infections were ruled out. Ferritin serum levels quickly decreased to 91 ng/ml. We hypothesized that the jaundice may be directly related to the Exjade treatment, even though no evidence was present in literature. Therefore, treatment was stopped and a complete remission of jaundice was observed. At 1 years since the stop therapy, the ferritin serum levels were still under 1000 ng/ml and the patient is still without any chelation therapy. It is unclear if jaundice was related to a minor liver damage, glucuronidation defect or an increasing microhemolysis. Considering a possible preferred free iron chelation, we suggest that Exjade should be carefully used in transfusion-dependent patients without parenchimal iron overload. Further follow up of this patient and possibly of others with the same clinical and biochemical characteristics are necessary for the correct and possibly tailored management of the treatment.

PU005

SEVERE INFECTION IN LOW RISK MYELODYSPLASTIC SYNDROME. SPONDYLODISCITIS: AN EMERGENT INFECTIVE COMPLICATION?

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Myelodysplastic syndromes (MDS) are associated with a risk of severe infections, but spondylodiscitis is rare and often recognized and treated too late. We present the case of a 82-yr-old male affected by MDS, subtype refractory anemia with ring sideroblasts, IPSS low, IPSS-R intermediate, admitted because worsening of general conditions, weight loss and back pain on November 2016. From 2008 to 2016 the disease was stable, no significant infections were registered, patient was treated initially with high dose erythropoietin and after with transfusion therapy and iron chelation. On August 2016 he presented fever, chest CT scan showed millimetric parenchymal infiltrate. Blood coltures from peripheral and central line were performed. Treatment with empiric broad-spectrum anti-bacterials and anti-fungals was started and patient conditions improved. But central line cultures showed the presence of Blastoschizomyces capitatum. We administered therapy with voriconazole for 2 weeks obtaining complete clinical and radiological resolution. In history no other infective episodes or microbiological findings. At admission peripheral blood showed Hb 8.3 g/dl, white blood cells 2.4x109/l with 1.6x109/l neutrophils and 213x109/l platelets, serum ferritin 3954 ng/ml, C-reactive protein 2.8 mg/l, peripheral blood smear highlights hypolobulated neutrophils, pseudo Pelger-Huet cells, clumping chromatine, no circulating blasts.

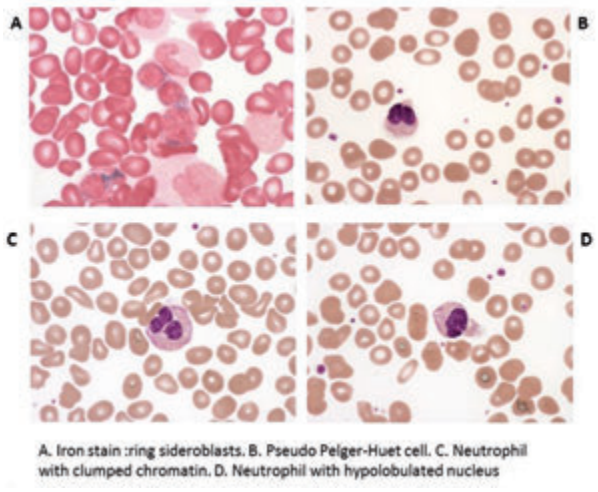

Figure 1. Blood Smear HP 500x.

Considering clinical conditions and back pain we ordered a spinal MR that showed arthrosis at D3,D4,L5,S1 with associated bulging discs. General conditions were worsening despite hematologic stability, negativity of new blood cultures and galactomannan and absence of fever or other infection signs. For this reason we research a second tumor by CT scan total body and neoplastic markers but all resulted negative. Finally the PET-CT scan showed hypercaptation of D4 body in March 2017 and a dorsal MR evidenced spondylodiscitis. The vertebral biopsy excluded presence of neoplastic cells or fungal hyphae, coltural studies were negative, maybe due to empirical therapies. Patient was treated with ceftriaxone 2 g/day for 3 weeks and associated prophylactic fluconazole, obtaining complete symptoms remission and weight improvement. To our knowledge, only one case report of spondylodiscitis in low risk MDS was described. This severe opportunistic infection may be related to lymphocyte deficiency often aggravated by iron overload and impaired granulocitc fuction.

PU006

FIRST CASE OF DER(3)T(3;8)(Q?;Q23.3) WITH PARTIAL GAIN 8Q INVOLVED IN UNBALANCED TRANSLOCATION OF ACUTE PROMYELOCYTIC LEUKEMIA WITH EXTRAMEDULLARY EAR RELAPSE

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Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia characterized by fusion of PML and RAR genes as a result of t(15;17)(q22;q12). APL is now one of the curable hematological malignancies thanks to molecularly targeted therapies based on all-trans retinoic acid (ATRA) and arsenic trioxide (ATX). Extramedullary (EM) relapse is a rare event in APL, ear involvement being even more infrequent, with only 6 cases so far described. About 30-35% of patients with newly diagnosed APL have additional cytogenetics abnormalities, whose prognostic significance is still controversial. The most common additional aberration is trisomy 8 or partial gain 8q. Three of our six last cases of APL showed an amplification of long arm chromosome 8: one patient had trisomy 8, one isochromosome (8q) and one partial 8q trisomy involving the region q23.3-24.3. Among these patients, we deeply investigated a 41-year old man with partial 8q trisomy. This patient was admitted to our Institute on July, 2014, with diagnosis of APL variant. The PML/RAR fusion mRNA and gene were detected by RT-PCR and FISH, respectively. Bone marrow (BM) karyotype was interpreted as: 46,XY,t(15;17)(q24;q21),der(3)t(3;?)(q?;?) or add(3)(q?). After achieving complete remission with AIDA 2000 protocol followed by three consolidations cycles, the patient developed EM isolated relapse in the auditory canal on February, 2015, with FISH for t(15;17) positive on ear biopsy and negative in BM. To better characterize the cytogenetic aberrations on BM, we also performed chromosomal microarray analysis by using Infinium CytoSNP-850K (Illumina). This analysis showed amplification of long arm of chromosome 8q23.3-q24.3. Moreover, two-color FISH with painting probes for whole chromosome 8 and 3 was used to define the nature of derivative (3); interestingly, results showed a new additional abnormality der(3)t(3;8)(q?;8q23.3). Stable complete remission was achieved after ATX treatment. In conclusion, we report here the first case of APL harboring der(3)t(3;8)(?;q23.3), leading to a partial 8q trisomy at diagnosis, with following extramedullary relapse. These findings support a pathogenic importance of region 8q23-24 and suggest the need of the comparative analysis of structural rearrangements usually involved in secondary abnormalities of APL. We are currently investigating this alteration at molecular level, aiming to identify novel fusion genes potentially involved in these cases.

PU007

POLYCYTHEMIA VERA, ACUTE PULMONARY EMBOLISM, SPLANCHNIC VENOUS THROMBOSIS: A CASE REPORT

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Introduction: Erythrocytosis is a clinical condition characterized by the increase in the red cell mass estimated by haematocrit and haemoglobin. Early diagnosis of this hematological condition can improve the prevention of thrombotic events as acute pulmonary embolism (PEA) and splanchnic venous thrombosis. PEA is a relative common cardiovascular emergency. PEA is a difficult diagnosis that may be missed because of non-specific clinical presentation. The initial symptoms of PEA and splanchnic venous thrombosis may include dyspnea, painless and abdominal pain. We report a 61-year-old man with abdominal pain, dyspnea and erythrocytosis. *Methods:* A 61-year-old man with history of surgery for carcinoma of the larynx was admitted to our hospital for abdominal pain, dyspnea, presence of hypoechoic lesions in the IV hepatic segment showing a roughly triangular shape with the base facing the splenic capsule. Color doppler US showed an area with no blood supply. Abdominal CT scan confirmed a splanchnic venous thrombosis with splenic infarction. Thoracic spin CT scan detected a PEA occluding pulmonary arterial bed. Erythrocytosis was diagnosed by complete blood count, according to the International Committee for Standardization in Haematology Criteria. Haemoglobin concentration was 18,2 g/dl; haematocrit was 50%. Bone marrow aspirate showed increased number of megacariocytes, with dysplastic features. The patient was positive to JAK2V617F mutation and fulfilled the WHO criteria for Polycythemia Vera (PV). Histology of bone marrow prompted the diagnosis of PV. *Results:* Treatment with enoxaparin 1 mg/Kg once daily was started. *Conclusions:* Thrombotic complications can be the major cause of morbidity and mortality as splanchnic venous thrombosis and PEA in erithrocytosis patients. Erithrocytosis is often occasionally diagnosed. When symptoms are present, they can be ascribed to the increase in the red cell mass, which can lead to hyperviscosity syndrome, or to an underlying disease. Arterial thrombosis is often observed when haematocrit value is over 55%. Moreover, clinical manifestation of erithrocytosis can be due to splanchnic and hepatic vein thrombosis with Budd-Chiari syndrome. Vascular complications, such as pulmonary embolism (PEA) can also occur in erithrocytosis patients. Early diagnosis of this hematological condition can improve the prevention of thrombotic events as acute pulmonary embolism (PEA) and splanchnic venous thrombosis.

PU008

PERICARDIAL EFFUSION AFTER SEPTIC FEVER DURING NEUTROPENIA POST CHEMOTHERAPY IN PATIENTS WITH ACUTE LEUKEMIA: EXPERIENCE OF A SINGLE HEMATOLOGY UNIT

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Pericardial effusion (PE) could develop in patients with cancer (lung, breast, lymphoma, leukemia) result from obstruction of the lymphatic drainage of the heart, frequently to infiltration of tumoral disease. In acute leukemia PE have been reported in 15-17% as part of leukemic disease process. In our study we analized PE in acute leukemias post infective. We reviewed 100 patients with acute leukemia (6 lymphoid and 94 myeloid) between august 2013 and march 2017 admitted to our unit of hematology treated with hypomethylant agents (41 patients for old age and/or comorbidities) or intensive chemotherapy (59 patients). All patients performed at least one echocardiogram before each chemotherapy cycle and during aplasia post therapy if symptoms/signs or cardiologists recommend. The cardiologist during echocardiogram examination assigned a grade between minimal(1), small(2), moderate(3) to large (4) to the PE detected. In 5 patients(5%) we discovered a PE of grade 1-2 and were monitored until resolution(1-3 months range). The main features of those patients were: median white blood cells at onset of leukemia (1000; range 600-1500), 100% acute myeloid leukemia with ELN adverse risk cytogenetic or molecular stratification and age <60 (range 52-60 years) and PE occurred after septic fever during neutropenia post chemotherapy. Those patients showed fever,dyspnea, chest pain, disconfort, easy faticability or sweating and all patients with PE blood colture positive(2 for E. coli, 1 for Enterobacter, and 2 patients for S. epidermidis). The patients required cardiological therapy with not steroid antinflammatory, colchicine and antimicrobic. No PE cases were found in patients treated with hypomethylant agents or lymphoid acute leukemia. Few study analized PE related to infections causes in acute leukemia and could be interesting to know the impact on survival and recovery from sepsis complicated with PE in a large a number of patients.

PU009

IBRUTINIB EFFICACY AGAINST RELAPSING CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) IN A PATIENT WITH PROLONGED REMISSION OF RICHTER'S SYNDROME

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Introduction: Ibrutinib is a BTK inhibitor that have shown remarkable efficacy in CLL, however little is known about its relationship to Richter's syndrome (RS). We report a case of Ibrutinib efficacy against CLL in a patient with prolonged remission of an aggressive lymphoma. Case report: A 73 years old man was diagnosed with CLL in 2003. Biological findings at onset included: absent ZAP70 and CD38 expression, mutated IgVH and subclonal NOTCH1 mutation. Patient was treated with FCR in 2008, resulting in partial response. In 2013, he presented with abdominal pain due to intestinal obstruction. A biopsy of a large stenotizing abdominal mass showed infiltration of DLBCL. Immunoglobulin gene rearrangement analysis indicated that the aggressive lymphoma was not clonally related to the underlying CLL and NOTCH1 mutational burden was lesser than diagnosed CLL. Patient was treated with 6 cycles of R-CHOP obtaining a complete response. 3 years later he presented a CLL progression with worsening lymphocytosis, anemia, thrombocytopenia, increased splenomegaly and lymphadenopathies. PET-TC scan excluded pathological uptake and FISH analysis showed deleted 12q. He started Ibrutinib achieving WBCs normalization, increased haemoglobin and platelets levels, disappearance of lymph nodes and reduction in spleen size. Therapy was well tolerated with no evidence of RS at 12 months' follow-up. *Discussion:* Data on the contribution of new CLL therapy to RS development are still controversial. In the initial studies of Ibrutinib in CLL the percentage of RS was at the upper limit of the incidence rate described in specific clinical trials. However, these patients were heavily pre-treated and had high-risk disease features. Additionally, many of transformation events developed shortly after starting therapy suggesting unrecognized RS likely preexisted treatment initiation. Conversely, RS was a rare event in patients treated with ibrutinib in recent trials. Because of a lack of efficacious standard treatment options, the efficacy of Ibrutinib was tested on RS demonstrating the possibility of transient disease control. *Conclusions:* This report indicated that Ibrutinib is effective and safe against CLL in a patient with sustained remission of the aggressive lymphoma and was not associated with RS recurrence, despite the patient was heavely pre-treated and presented high-risk biological and clinical disease features associated with CLL transformation.

PU010

BENDAMUSTINE TREATMENT MAY INDUCE LONG-LASTING COMPLETE HEMATOLOGIC RESPONSE IN RELAPSED/REFRACTORY T-LARGE GRANULAR LYMPHOCYTE LEUKEMIA

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Large granular lymphocyte leukemias (LGLL) are chronic clonal lymphoproliferations of effector memory cytotoxic CD3+CD57+CD56- T cells. We report two cases of refractory/relapsed T-LGLL treated with bendamustine. The first case was a 73-year-old woman who received diagnosis of T-LGLL in 2003 in other institution where she was treated in 2008 with low-dose oral MTX and prednisone (PDN) and then 6 months later with oral CTX (100 mg/day) and PDN due to anemia and thrombocytopenia. In August 2012, the patient was referred to our institution for transfusion-dependent severe anemia and neutropenia, where was confirmed the diagnosis of T-LGL leukemia CD3+CD8+ CD7+CD5-CD4-CD56-TCR +. After 8 months of no clinical response to Cyclosporin A (CyA), the patient started a salvage therapy with bendamustine (70 mg/m2 for 2 consecutive days every

28 days). After the first bendamustine course, our patient experienced a rapid hemoglobin improvement and transfusion independence, achieving complete remission (CR) within three months. CR was maintained for 20 months, when the patient showed again anemia; for this reason, a second course of 4 cycles of bendamustine was administered, without any clinical toxicity. At last follow-up, 18 months after the end of therapy, the patient is still in CR. The second case is a 72-year-old man admitted to our department in October 2013 for severe anemia associated with atypical circulating and marrow LGLs CD3+CD8+CD2+CD7+CD4-CD5-CD56-TCR +. This patients, four months after CyA and PDN therapy, for persistence of transfusion-dependent anemia and the worsening of neutropenia (0.29x109 cells/L) required a second line therapy. Bendamustine was administered for a total of 6 cycles at 70mg/m^2 for 2 days every 28 days. CR was achieved after 6 cycles of bendamustine and, after that, the patient no longer needed transfusions or Erythropoietin administration. After 32-month of follow-up, the patient was still in CR. Athough these efficacy and safety of bendamustine in the treatment of elderly relapsed/refractory T-LGLL patients require further validation in prospective randomized studies, our case series suggest that bendamustine as single agent or in combination should be considered as a feasible second-line option for relapsed or refractory T-LGLL.

PU011

REACTIVE FOLLICULAR HYPERPLASIA DURING DASATINIB TREATMENT: AN UNDERESTIMATED ADVERSE EVENT

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Dasatinib (DAS) shows a distinct toxicity profile among which a previously unrecognized adverse event (AE) is represented by persistent lymphadenopathy with reactive follicular hyperplasia (FLH). The latter is a common cause of lymphadenopathy and encompasses a constellation of morphologic and immunophenotypic features. Recently, lymphadenopathy with morphologic features of reactive FLH has been described in two small series of long-term DAS-treated CML patients. However, only in a few patients complete morphologic and immunophenotypic features of this AE was reported. Herein, we describe 2 cases of patients with chronic phase (CP)-CML who presented with unexplained lymphadenopathy after front-line therapy with DAS. Case 1: in October 2012 a 30-year-old man was diagnosed with CP-CML and he was treated with DAS front-line, rapidly obtaining a major and subsequently a deep molecular response. DAS was well tolerated but approximately after 48 months he acceded to our Hospital because of the appearance of bilateral cervical lymphadenopathy. Case 2: in April 2016 a 49-year-old man was diagnosed with CP-CML and he was treated with DAS front-line. The drug was well tolerated and after 12 months of therapy he obtained a major molecular response. Nevertheless, he acceded to our Hospital because of the appearance of a swelling at the angle of the left mandible and concomitant bilateral cervical, preauricular and sovraclavear lymphadenopathy. For both patients no generalized lymphadenopathy was noted and no constitutional symptoms were reported. As screening for active viral infection was negative and no signs of local or systemic infectious disease were detected, an excisional biopsy was performed, showing in both cases enlarged lymph nodes with overall preserved architecture, follicular and paracortical hyperplasia with a reactive phenotype (BCL2-/BCL6+/CD10+), a predominance of small CD3+, CD5+ T-lymphocytes mixed with much rarer enlarged CD45+, CD30-/+, CD15-, CD20+/- lymphocytes with blastic morphology. *In situ* hybridization for EBV-encoded RNA was negative. A diagnosis of FLH was made, ruling out an extramedullary blastic transformation of CML. Then, both patients definitely discontinued DAS, achieving clinical resolution of lymphadenopathy. Therefore, we support the importance of a careful clinical examination of lymph nodes in all DAS-treated CML patients and provide additional information concerning the real incidence and the correct management of this AE.

PU012

EFFECTS OF GENERIC IMATINIB ON A COHORT OF ITALIAN CML PATIENTS

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Background: Generics of Imatinib mesylate have recently been approved in Italy for the treatment of patients (pts) affected from chronic myeloid leukemia (CML). However, efficacy and safety of imatinib generics were not studied and reported on larger cohort of pts in Italy yet. *Aim:* The aim of this study is to evaluate the short-term clinical outcome, expressed as variation in quantitative polymerase-chain-reaction(PCR), of pts affected from CML treated in our institute, who switched from branded to generic imatinib. *Patients and Methods:* This is a retrospective analysis of 11 pts affected from CML in chronic phase with stable disease who switched from branded to generic imatinib from January to March 2017. We analyzed the variation of quantitative PCR values, considering BCR/ABL gene copy number/10000 ABL gene copy corrected by the International Standard (IS). Two PCR values were considered before the switch: one (pre2) obtained 3-6 months before the switch and one (Pre1) 0-2 months before it. Wilcoxon non parametric test for individual paired data was used to compare the average number of pre switch copies with the number of post switch copies. A 5% significance level was considered for two sided test. *Results:* PCR determinations after change to generic imatinib (Post) were performed after a median switch duration of 47 days. The mean value of the two PCR performed before the switch was 0,985 x 10(-4) (SD 0,930x10(-4); 95% CI 0,655- 1,315x10(-4)), while the mean value after the switch was 0,818x10(-4) (SD 0,950x10(-4); 95% CI 0,481-1,156x10(-4)). Mean PCR value after switch was reduced by 0,167 compared to gene copy number observed before the switch. No statistically significant difference was found between these values (p= 0,0696). Subjective adverse events reported by pts after the switch included diffuse muscular cramps (5/11pts), conjunctive hyperemia (3/11), fatigue (2/11), nausea, vomiting and diarrhea (1/11). All events were grade I-II. Three pts expressed concerns about the efficacy of generic imatinib. *Conclusions:* Our preliminary data obtained in this small cohort of pts followed for a limited period of time suggest that generic imatinib does not have deleterious effects on CML control. Extensive discussions with pts are useful to clarify doubts and fears about generic imatinib.

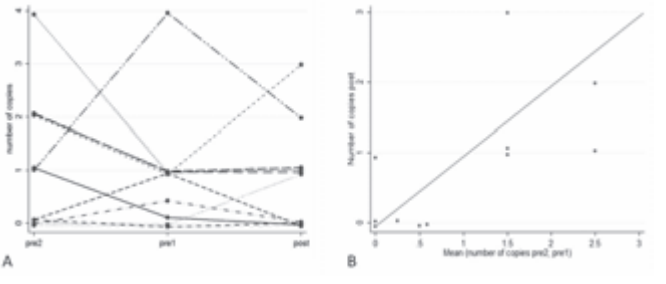

Figure 1. A. Spaghetti plot with jitter, showing the patient trajectories in time. The x axis refers to the time scale ("post" the time of PCR post change to generic imatinib, pre 2 and pre-1 the two PCR before the change). The y axis refers to BCR/ABL gene copy number out of 10000 ABL gene copy. B. Scatter plot contrasting gene copy number before (x axis, average of two values) and post change (y axis). Points beyond the bisector represent an increase in gene copy number after treatment witch.

PU013

MANAGEMENT OF DASATINIB THERAPY SIDE EFFECTS IS CHEAPER THAN IMATINIB MULTICENTRIC RETROSPECTIVE STUDY

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Patients and methods: This study is a retrospective multicentric study. 13patients, M/F:8/5, median age75(R60-78) received imatinib 400mg/day and 15patients, M/F:9/6, median age 68(R50-72) received dasatinib 100mg/day. During an observation period of 7 years 3 imatinib patients showed 1acute heart failure and hepatotoxicity G4, 1 chronic diarrhea G3, 1 acute recurrent abdominal pain G4 and 3 dasatinib patient showed 1 osteomuscular pain G4, 1 gastrointestinal bleeding G4, 1 pleural effusion. For each patient, the overall cost of side effects management during the entire follow-up period was calculated. This cost was then divided by the days of hospital admission, in order to give an average daily patient treatment cost. Then in each group the median of average daily costs was performed. Cost for each diagnostic and therapeutic intervention was considered in conformity of Italian National Health Service. *Results:* In imatinib group the median days of hospitalization were 30(R7-60), with a median daily expense of 366€(R330-461),a median complication management cost of 10980€ and a median hospitalization cost for each patient of 22500€. In dasatinib group the median days of hospitalization were15(R3-30), with a median daily expense of 275€ (R195-370),a median complication management cost of 4125€ and a median hospitalization cost for each patient of 11250€. For each patient the median saving in dasatinib side effect management respect to imatinib group is 11250€ for hospitalization and 6855€ for complication management. In dasatinib group the saving is about 90€/day. All imatinib patient with side effects changed therapy with a IInd generation TKI with an increase in follow-up test expense of about 13€/day for the first 6 months. *Conclusions:* Management of side effects of dasatinib seems to be cheaper than imatinib. These data need confirmation on a larger cohort of patients.

PU014

SAFETY AND EFFICACY OF PONATINIB IN A CML PATIENT WITH HISTORY OF CARDIOVASCULAR EVENTS

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Background: Ponatinib is a potent second generation TKI approved for resistant/refractory CML or in case of T315I mutation. Serious cardiovascular events have been reported with full-dose ponatinib and this may limits its usage in elderly patients or in those with CV comorbidities. Case description A 71-year old man with history of coronary artery disease was diagnosed with CP-CML Sokal risk intermediate in 2006. He started imatinib obtaining a MMR after 12 months but it was discontinued after 2 years due to severe gastrointestinal toxicity and dasatinib was introduced. After 12 months MMR was retained but recurrent pleural effusions developed; ongoing treatment was first reduced and then definitely stopped in november 2009, when nilotinib was started. After 4 years of efficient and well tolerated therapy, an acute limb ischemia occurred which required urgent revascularization. In 2015 a second lower limb thrombotic event occurred so nilotinib was definitely stopped and we opted for a rechallenge with imatinib. However the patient lost his molecular response and BCR/ABL increased up to 70% IS; mutational analysis did not demonstrate ABL1 mutations and we opted for bosutinib, based on its favorable toxicity profile. After 3 months BCR/ABL level was 84% IS and the patient progressed toward AP-CML. Despite concerns about its CV toxicity, reduced-dose ponatinib (15 mg OD) was started. Ponatinib revealed highly effective and well tolerated and BCR/ABL level was profoundly reduced to 1.01%, 0.56% and 0.16% IS after 3-, 6- and 9-months, respectively, with no remarkable toxicity. *Discussion:* Patients who develop resistance to one or more TKI have a poor outcome because of progression to AP or BP-CML; the PACE trial reported that a large portion of such patients can still achieve a molecular response with high-dose ponatinib. However, recent reports of dose-dependent CV toxicity have raised concerns about long-term sequelae of this molecule. In our case we used for the lowest effective dose of ponatinib because of recurrent thrombotic events occurred during previous TKI therapy. Nevertheless, the treatment revealed highly effective and there were no other adverse events during follow-up. This report supports the hypothesis that, particularly in patients with CV comorbidities, lowering ponatinib dose might reduce its toxicity, while retaining its efficacy in terms of disease control.

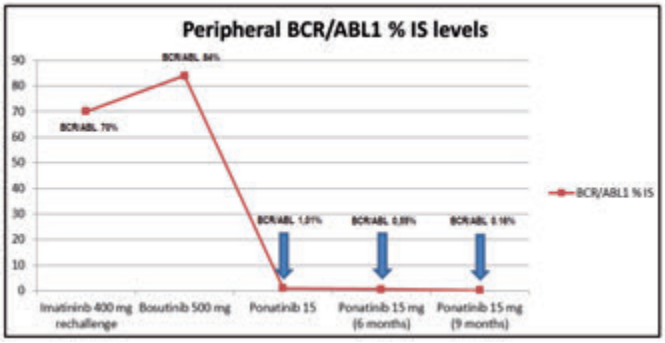

Figure 1. Peripheral BCR/ABL1 transcript levels at 3 months from the start of Imatinib, Bosutinib and Ponatinib; and after 6 and 9 months on Ponatinib.

PU015

DECITABINE AS THERAPY FOR A PATIENT WITH ACUTE MYELOID LEUKEMIA SECONDARY TO MYELODYSPLASTIC SYNDROME TREATED WITH AZACITIDINE: A CASE REPORT

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UOC Ematologia - Ospedale "A. Perrino"

Azacitidine (Aza) and decitabine (Dec) are hypomethylating agents (HMA). Aza is approved for the treatment of myelodysplastic syndromes (MDS) with Intermediate-2 or High IPPS risk and acute myeloid leukemia with myelodysplasia related changes (AMLWMRC) with bone marrow blasts from 20 to 30% not eligible for standard chemotherapy. Dec is approved for the treatment of AMLWMRC not eligible for standard chemotherapy. Few data are available on sequential use of Aza and Dec. We report a case of a 80 years old female patient, diagnosed with a IPSS high risk MDS (Refractory anemia with excess of blasts, RAEB-2) transfusion dependent with a bone marrow blasts of 12%, thrombocytosis and splenomegaly at diagnosis. She was treated with Aza (75mg/mq/die d 1-7 every 28 days) for 11 cycles and hydroxyurea, keeping stable disease. Subsequently she developed a AMLWMRC with bone marrow blastosis of 30% and high leukocytosis in peripheral blood (about 200000/µl, including 10% of blasts). Considering patient characteristics, we started treatment with Dec (20 mg/mq/die d 1-5 every 28 days) associated to hydroxyurea. After 4 cycles the bone marrow blasts decreased to 10%. The patient was treated for 10 cycles with stable hematological features and died for pneumonia as complications of a previous obstructive chronic bronchitis. This case report showed the benefit derived from the treatment with Dec in a AMLWMRC secondary to MDS previously treated with Aza. Future trials needed to evaluate sequencing with HMA in this setting of AML patients.

PU016

DECITABINE AS TREATMENT IN A PATIENT WITH ACUTE MYELOID LEUKEMIA AND COMPLEX KARYOTYPE WITH DEL(7Q): A CASE REPORT

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In acute myeloid leukemia (AML), the karyotype of the leukemic cells is the most powerful predictor of treatment outcome. Approximately 10-12% of cases of *de novo* AML have a complex karyotype (≥3 acquired chromosome aberrations) with a median overall survival of about 7 months. Decitabine (Dec) is an hypomethylating agent approved for the treatment of AML in patients not eligible for standard chemotherapy. We report a case of a 68 years old patient, male, with AML with complex karyotype included del(7q), NPM and FLT3 negative. The patient had severe anemia transfusion dependent, moderate leukocytosis and moderate thrombocytopenia. The bone marrow blastosis was about 60%. Due to severe cardiopathy the patient was considered unfit for high dose chemotherapy and so we started Dec 20 mg/mq/die d 1-5 every 28 days. After 4 cycles the bone marrow blastosis decreased to about 25%, hemoglobin level increased to about 10 g/dl, platlets and leukocytes reached normal range. After 8 cycles, a partial remission was obtained with a residual bone marrow blastosis of

10%. Cytogenetic analysis revealed the persistence of complex karyotype, included del(7q). The patient is still in treatment with Dec (10 cycles, Overall Survival 12 months) with stable levels of hemoglobin, leukocytes and platlets. Treatment is well tolerated. This case report shows that Dec is an effective and safe option for the treatment of unfit AML patients with complex karyotype, demonstrating an increased OS compared to the data available in this setting. Moreover these data suggest the importance of continuous treatment with Dec, despite the absence of a complete remission.

PU017

REVERSIBLE SYMPTOMATIC PERICARDITIS INDUCED BY ALL-TRANS RETINOID ACID (ATRA) AND ARSENIC TRIOXIDE (ATO) DURING INDUCTION-TREATMENT FOR ACUTE PROMYELOCYTIC LEUKEMIA (APL)

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Background: ATRA and ATO are the current mainstay drugs for the treatment of standard-risk APL.Pericardial effusion in patients undergoing ATRA-ATO treatment has been reported as mainly associated to APL differentiation syndrome (APL-DS) or fluid retention.Here, we report the unique case of a patient who developed acute isolated pericarditis with pericardial thickening during ATRA-ATO induction treatment for APL. *Report:* A 39-year-old woman was admitted to our hospital with methrorragia, gum bleedings and diffuse bruising.EKG, Chest X-Ray and echocardiography were normal.Blood tests revealed pancytopenia and altered coagulation function, and bone marrow examination established diagnosis of APL with PML/RARa rearrangement. Induction therapy with ATRA and ATO was started.On day 13, the patient experienced intense chest pain, worsening in the supine position and upon inspiration, whilst improving by knee-chest position. Physical examination revealed pericardial rub. EKG showed sinus tachycardia. Echocardiography showed hyperecogenicity of pericardial layers with negligible pericardial effusion.Infectious etiology of pericarditis was ruled out.With the suspect of APL-DS, ATRA and ATO were temporarily discontinued while i.v. Dexamethasone was started. Because of the reappearance of signs and symptoms of the disease, therapy was reinitiated, and few days later, on day 27, chest pain reappeared. Echocardiography highlighted thickening of pericardial layers without further abnormalities. Pain-control therapy was markedly potentiated to ensure prosecution of ATRA-ATO therapy for other 10 days until the bone marrow examination revealed achievement of complete hematologic remission. Strikingly, all symptoms disappeared shortly after ATRA-ATO administration ceased and did not appear again during the following consolidation cycles. *Conclusions:* The timing and the clinical course instill the doubt of pericarditis etiology. APL-DS, idiopathic/viral pericarditis, and ATRA-ATO-induced pericarditis were considered. The absence of APL-DS defining features (dyspnea, hypotension, pleural and pericardial effusion, weight gain), the lack of any viral findings, the disease remission during the events, the cessation/relapse of symptoms and Echo-signs with withdrawal/resumption of ATRA-ATO therapy really foster the diagnosis of drug-induced pericarditis. At the best of our knowledge, this is the first report of drug-induced pericarditis during ATRA-ATO treatment for APL.

PU018

A CASE OF BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM SUCCESSFUL TREATED WITH HIGH-DOSE LYMPHOID-LIKE CHEMOTHERAPY FOLLOWED BY AUTOLOGOUS HEMATOPOIETC STEM CELL TRANSPLANTATION

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Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare aggressive hematologic malignancy due to clonal proliferation of resting plasmacytoid dendritic cells (pDCs) with a poor prognosis, classified among myeloid neoplasms in the 2016 WHO revision. This disease is characterized by isolated skin lesions and less frequently bone marrow (BM) involvement at diagnosis, but followed invariably by systemic dissemination with BM and extramedullary infiltration. The optimal treatment strategy for BPDCN has not yet been established, being sometimes treated as an acute myeloid leukemia, sometimes as an acute lymphoid leukemia or a myelodysplastic syndrome. Allogeneic hematopoietic stem cell transplantation (HSCT) with myeloablative conditioning in first complete remission (CR1) has shown encouraging results for eligible patients. We report a case of BPDCN with classic presentation treated with an ALL-like schedule followed by autologous HSCT (auto-HSCT). A 48-year-old woman was admitted in our institution in March 2014 for multiple erythematous plaques and violaceous nodules on legs' surfaces, treated during the previous year as erythema nodosum with topical steroid and colchicine with worsening of clinical condition. Blood counts were normal, no hepatosplenomegaly and lymphadenopathy were detected. A PET showed a SUV max of 2.8 on left leg at nodular lesions, MRI excluded the infiltration of the muscle compartments. A punch biopsy showed a diffuse dermis and hypodermis infiltration by medium-size, ovalar cells, with high nuclear/cytoplasmic ratio, open chromatin and inconspicuous nucleoli. The immunohistochemical stains showed positivity for CD4, CD56 and CD123, Ki-67 of 80% and negativity for T-cell, B-cell, and myelomonocytic cell lineage-associated markers. BM and peripheral blood immunophenotyping showed 1% and 1.2% of CD33+ CD123+ blastic pDCs. These results were consistent with the diagnosis of BPDCN. The patient, was treated with 4 courses of hyper-CVAD/MTX-Ara-C, achieving complete remission (CR). Afterwards, having no matched related donor, the patient underwent to auto-HSCT preceded by a conditioning regimen with busulfan-melphalan. At present, 36 months after diagnosis, she is still in CR. Our case report provides further evidence that high-dose ALL-like therapy followed by auto-HSCT is a valuable therapeutic option in BPDCN patients without matched related donors.

PU019

COPY NUMBER VARIANTS SIGNATURE IN TWO PATIENTS WITH RELAPSED ACUTE PROMYELOCYTIC LEUKEMIA

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Introduction: Nowadays, Acute Promyelocytic Leukemia (APL) is a disease entity with a very high rate of cure and an estimated 2-year overall survival of 97%. Early death, rather than resistant disease so common in all other subtypes of AML, has emerged as the major cause of treatment failure, and relapse is a very rare occurrence. *Methods:* We collected data of all the APL referred to our institution from 2014. Within 23 patients, we encountered 20 new diagnosis and 3 relapse of APL. A patient was excluded from the analysis because she relapsed for very poor therapy adherence. We analyzed blasts in samples obtained from Bone Marrow with Single Nucleotide Polymorphisms Array Cytoscan HD®. *Results:* We compared copy number alterations in both relapsed patients with alterations detected in the pool of 20 newly diagnosed APL and we found specific signatures of CNVs for each patient. There were no events that were detected in both patients. There were several copy number alterations related to each patient: the first patient presented gains of ROBO2, GRIP1, CTNNB1, SOX6, PBX1, GRIK2, CDKAL1 and loss FAF1, CREBBP, SBF1; the second patient presented the gains of ROBO1, MAPK10, CADPS2, APBA1 and the loss of GRIP1, MYB. Subsequently we focused our attention on ROBO genes and GRIP1 gene because they were alterated in both relapsed patients: ROBO proteins are associated to K channels; GRIP1 is involved in various critical functions (for example in androgen receptor binding, beta-catenin binding, glucocorticoid receptor binding, and it i salso a regulator of glutamate metabolism, a well-known pathway in LSC). Especially GRIP1 data demands for further epidemiological and functional testing. *Conclusions:* APL relapse is a very rare entity, and it is announced to become rarer with the advances in first line therapy. Molecular characteristics are hard to analyze without an effort to collect and bank samples together from multiple institutions. Since relapses, especially relapses out of follow-up period, represent a sudden life-treating condition for patients, to predict patients at higher risk of relapse (a major

health problem in APL patients) we selected two candidate genes that could be involved in pathways favoring relapse; by the analysis of these two genes at the diagnosis of APL we could establish a different and strict follow-up program for patients with these alterations. *Acknowledgements:* ELN, AIL, AIRC, PRIN, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project, HARMONY project.

PU020

DECITABINE TREATMENT FOR ACUTE MYELOID LEUKEMIA IN ELDERLY NON-FIT PATIENTS: A SINGLE INSTITUTION EXPERIENCE

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Background: Decitabine (DCT) is approved for elderly patients (pts), with acute myeloid leukemia (AML) not eligible for intensive chemotherapy. Treatment of these patients remains a major challenge. Aim of our study was to evaluate retrospectively our population of elderly frail AML treated with DCT as a first-line treatment. *Methods:* From January 2014 to April 2017, we diagnosed AML in 19 elderly frail patients. Eligible for therapy were 10, median age 79 years (range, 67-83), pts received at least one cycle of DCT (20mg/m^2/d i.v. for 5 d every 4 weeks), as first-line therapy. Eight pts were AML *de novo* and two were secondary to myelodysplastic syndrome. Four pts had normal karyotype, one pt t(9;22), one trisomy 11 and one FLT3-ITD mutation, the remaining were not evaluable. Results: The median number of DCT cy were 5 (range, 1-12). Two pts died early: one because of infection (1st cy), the other for hemorrhagic episode (2nd cy). Response to treatment was evaluable in 8/10 pts. A complete response (CR) or CR with incomplete blood-count recovery (CRi) was achieved in 5/8 patients (62.5%) after a median of six cy (range, 4-8). One patient obtained a partial remission (PR), one stable disease (SD) (both after 4nd cy) and one disease progression (2nd cy). Nine out of ten (90%) patients had grade ≥3 non-hematologic toxicity. The most common toxicity was infection, occurring in 8/10 pts. The total infective episodes during DCT treatment were 15, also multiple in the same pt: 10 bacterial pneumonia, 3 fungal pneumonia (Aspergillus) and two sepsis, one of these was fatal. All episodes occurred during neutropenia (median neutrophil count was 0.13x10^9/L). Furthermore, we recorded 2 hemorrhagic events and 2 cardiovascular. The median days of hospitalization were 52 (range, 20-128). The median time of observation was 200 days (range, 28-508). The median overall survival was 400 days. Among the 5 CR patients, 3 relapsed (60%) after 53-190-216 days, respectively; median duration of response in CR pts was 53 days (range, 30-216). *Conclusions:* DCT is perceived as a feasible treatment for older AML, however the efficacy and the reduced toxicity of this approach has been called into question into several study. In our experience infection complications, plays a major influence on the prognosis of these pts and the role of infective prophylaxis may improve prognosis together with an unexplored, at this time, strategy that would include a consolidation treatment.

PU021

HYPOMETHYLATING AGENTS FOR THE TREATMENT OF RELAPSED ACUTE MYELOID LEUKEMIA AFTER ALLOGENEIC BLOOD STEM CELL TRANSPLANTATION: A SINGLE CENTER EXPERIENCE

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Allogeneic blood stem cell transplantation (allo-SCT) is a potentially curative therapy for acute myeloid leukemia (AML), but relapse still represents the major cause of treatment failure. Hypomethylating agents (HMA) azacitidine (Aza) and decitabine (DAC) have been tested alone or in combination with donor lymphocyte infusions (DLI) in the post-transplant period with well-balanced profile of efficacy and toxicity. We aimed to retrospectively evaluate the safety and efficacy of HMA+/-DLI in a real-life cohort of AML patients (pts) relapsing after allo-SCT. It has been collected data of AML pts who underwent allo-SCT at our Institution in the last 5 years and subsequently received HMA as a salvage treatment for disease relapse or preemptively for loss of complete donor chimerism. Seven pts were identified. At the time of HMA treatment, median age was 57 years and median time between allo-SCT and HMA therapy was 10 months. Three pts received Aza subcutaneously: 2 at the standard 75mg/m^2 dose for 5+2 days and 1 with 100mg/m^2 for 5 days. The remaining 4 pts were treated with DAC 20mg/m^2 i.v. for 5 days. The cycles were repeated every 28 days. Six pts started HMA for morphological AML relapse, while 1 case for a sequential treatment after DLI for loss of complete donor chimerism. Median administered number of cycles was 2 (1–6). Treatment strategy included combination with DLI in 3 pts (2 in the DAC cohort, 1 in the Aza cohort); in 1 case sorafenib was associated to DAC/DLI. HMA was well tolerated with no grade 3/4 extrahematological toxicities or no occurrence of acute graft *versus* host disease (GvHD). Three patients had a clinically significant response, all receiving ≥4 cycles: 2 complete remissions in pts with morphological relapse (1 received DAC/DLI/sorafenib and 1 Aza 75mg/m^2) and a donor chimerism ≥50% in the patient who received Aza for the progressive loss of donor chimerism. The median overall survival from HMA treatment was 4 months; at the time of data collection responses were maintained in all 3 pts, while stable disease was documented in 2 pts having received the first 2 cycles. Two pts had died, 1 of AML progression and 1 of severe intestinal GvHD after Aza failure and a following salvage induction chemotherapy. Although arising from a limited number of pts, our real-life experience of HMA+/-DLI in AML relapsing after allo-SCT showed a general good safety profile and promising antileukemic activity.

PU022

ARSENIC TRIOXIDE INDUCED PERIPHERAL NEUROPATHY: PROSPECTIVE EVALUATION OF TWO PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA

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Arsenic trioxide (ATO) is highly effective in treatment of acute promyelocytic leukemia (APL). ATO most frequent side effects are well described, but less is known about ATO induced neuropathy. Two patients with Sanz's low risk APL were treated with ATRA/ATO as first line chemotherapy. We prospectically analyzed the occurrence and clinical course of ATO induced neuropathy, performing strict neurological evaluation using both the total neuropathy score, clinical version (TNSc, a validated measure for chemotherapy induced peripheral neuropathy) and neurophysiological assessment. Patients have been evaluated at baseline (before starting ATO/ATRA therapy), at the end of the induction phase, at the end of ATO/ATRA treatment and 1 year after the discontinuation of treatment. Baseline neurophysiology was performed at the end of induction phase. Patient 1 was 41-yr-old, and patient 2 was 53-yr-old; both were male. None of the patients had previous history of neuropathy, or known risk factors for peripheral neuropathy. Baseline TNSc was 0 (no clinical signs of neuropathy) in both patients. Neurophysiological evaluation performed after the end of induction cycle did not reveal signs of peripheral neuropathy in both patients. Patient 1 received 387 mg of ATO during induction, total 1040 mg. Patient 2 received 311 mg of ATO during induction, total 1188 mg. Both patients developed leg numbness during consolidation cycles. Patient 1 also developed hand numbness. TNSc at the end of therapy worsened in both patients; it was 1 in patient 1 and 3 in patient 2. Neurophysiology at the end of therapy detected sensory axonal neuropathy in both patients, more severe in patient 1. Both patients received full doses of ATO consolidation (0.15mg/kg/day for five days/week, on alternate months for total three months and tretinoin 2 weeks on 2 weeks off, continuously). During the first year of follow-up symptoms improved gradually in both patients, and both TNSc and neurophysiology 1 year after the end of consolidation cycle were consistent with full recovery. Both our patients developed sensory axonal neuropathy during ATO therapy. Symptoms and signs dramatically improved up to complete recovery during one-year follow-up. ATO-induced neuropathy in our 2 patients was mild and reversible, and clinically manifested only during

consolidation cycles. We are starting a multicenter prospective study to evaluate the occurrence, characteristics and risk factors of ATO-induced neuropathy in APL patients.

PU023

DONOR-TRANSMITTED TRIPLE-HIT LYMPHOMA IN A RENAL ALLOGRAFT RECIPIENT

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Donor cancer transmission (DCT) is a rare long-term complication of kidney transplantation that carries an estimated risk of approximately 0.05%. Diagnosis and management of these malignancies are difficult because of their rarity and the need for an individualized approach to treatment. CM is a 30-years-old man with a medical history significant for chronic kidney disease who underwent kidney transplantation in May 2015 with his mother as the donor. In July 2015, the donor presented with an abdominal mass diagnosed as a triple-hit lymphoma (THL), a subset of highly aggressive B-cell lymphomas characterized by the overexpression of MYC, BCL2 and BCL6 and high proliferative index. Initial staging with PET/CT and bone marrow biopsy showed widespread disease with no marrow involvement, while circulating tumor DNA (ctDNA) assay confirmed a clonal B-cell proliferation. Analysis of ctDNA was performed on recipient's plasma and marrow, but ctDNA levels were undetectable. In September 2015, the recipient developed a perigraft mass that was identified as a THL as well. The clinical presentation and the histochemical identity of the two THLs raised the suspicion that the same lymphoma had been transmitted from donor to recipient during the transplant procedure. Histology and immunohistochemistry showed identical findings in both donor and recipient. FISH recognized an XX pattern in both samples. Microchimerism analysis pointed out that the donor and the recipient THLs had identical profiles considering discriminant alleles and amelogenin (see Figure). Moreover, the recipient sample profile was significantly different from his own basal allelic profile obtained from peripheral lymphocytes at the time of the diagnosis therefore confirming DCT. The same clonal band was persistently detected by ctDNA analysis on recipient's plasma only after September 2015. The patient went on to complete the full course of 2 cycles of RCODOXM/IVAC and associated withdrawal of immunosuppression achieving a complete remission with negative ctDNA levels and preserving graft function. Follow-up surveillance by PET/CT scan and ctDNA analysis in April 2017 confirmed a persistent complete remission. In conclusion, we confirm the validity of ctDNA analysis as an adjunct to imaging for the management of patients with high-grade B-cell lymphomas and provide an example of feasibility of intensive chemotherapy with preservation of graft function in the setting of kidney transplant patients.

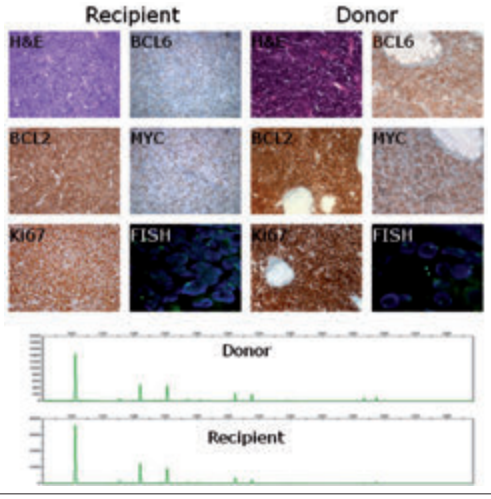

Figure 1.

PU024

CT IMAGING OF PRIMARY PANCREATIC LYMPHOMA

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UOC Ematologia, Radiologia, Anatomia Patologica

Introduction: Primary Pancreatic Lymphoma (PPL) is a rare disease, with several clinical features in common with the pancreatic ductal adenocarcinoma (PDAC). Our aims are: to evaluate imaging characteristics of PPL at CT basing upon a series of cases examined in a single Institution; to depict useful CT features to distinguish between PPL and PDAC. *Materials and Methods:* Five patients with histopathologically proven PPL were enrolled. CT examinations included: unenhanced scan, contrast-enhanced pancreatic phase and portal phase. Qualitative analysis included: tumor location; major peripancreatic vessels encasement; necrosis within the tumor; abdominal enlarged lymphnodes; fat stranding in the peripancreatic region; enlarged common bile duct or main pancreatic duct. The quantitative parameters were: neoplasm longest dimension, volume and tumor density. PPL imaging features were compared with PDAC characteristics reported in the Literature. *Results:* Histopathological diagnoses were: diffuse large B-Cell lymphoma (1/5), high grade B-Cell lymphoma NOS (3/5) and follicular lymphoma grade 3A (1/5). Qualitative analysis: 3/5 PPL were located in the pancreatic head, 1 in the tail and 1 involved the whole gland. In 4/5 cases celiac axis, superior mesenteric artery and superior mesenteric vein were encased; splenic vein and splenic artery encasement was depicted in one PPL. Only one patient presented necrosis within the tumor. Enlarged retroperitoneal lymphnodes were present in 3/5 cases; fat stranding was depicted in every patient. Common bile duct was dilated in 3/5 cases; main pancreatic duct enlarged in 2/5 patients. Quantitative analysis: Mean neoplasm longest dimension and volume were 12,8 cm (range 10-18 cm) and 605,4 cm3 (range 84,83-1126,94 cm3). Mean tumor density was 39,4 HU (range 32-49 HU) without contrast, 56,2 HU (range 43-71 HU) in the pancreatic phase and 67 HU (range 61-75 HU) in the venous phase. *Conclusions:* PPL usually manifests as a homogeneous lesion with delayed enhancement; peri-pancreatic fat stranding and enlarged lymphnodes are common. Compared to PDAC, PPL tends to be present larger dimension, vessels are encased but not infiltrated and main pancreatic duct is not dilated.

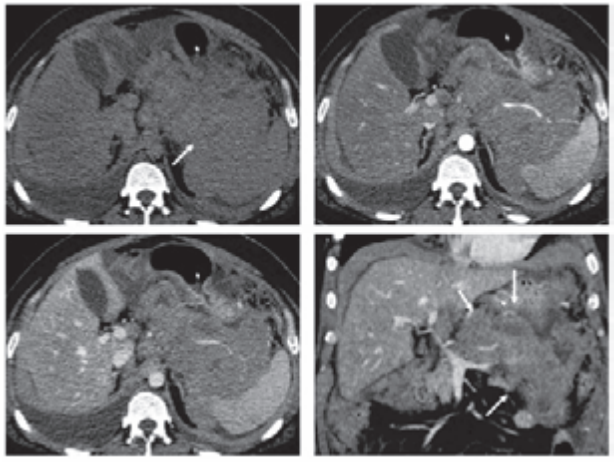

Figure 1.

PU025

INTRAVASCULAR LARGE B CELL LYMPHOMA: THE GREAT IMITATOR

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AOUP

Background: A rare type of diffuse large B-cell lymphoma,intravascular large B-cell lymphoma,primarily affects the middle-aged to elderly population,with poor prognosis. A 66 years old female,with no history of relevant pathologies,complained of fever associated with weakness,hyporexia and weight loss since June 2016.Chest and abdomen CT scan and PET-TB revealed no pathological findings.All the work up per-

formed excluded infectious or neoplastic causes. A diagnosis of suspected Still disease was made. The patient was given a high-dose systemic steroid therapy and anti-IL1 with fever resolution.Two months later subcutaneous, painful, nodular lesions suddenly appeared on breast, abdomen and limbs and were biopsyed. Ultrasound with contrast media exam of the lesions showed hypervascular pattern. The patient became aphasic and dyspnoic. Chest CT scan excluded pulmonary embolism. Brain CT scan and RMI showed no lesions. The patient was then sent for hematologic counseling for marked microcytic anemia, thrombocytopenia above 100000/mmc. Blood chemistry showed: increased inflammatory markers,presence of polyclonal gammopathy in accordance with the rheumatologic disease, CMV viremia, hypertriglyceridemia, increased IL2 receptor,hypoalbumineamia, hyponatremia. Marrow aspiration showed many istioreticolar cells with hemophagocytosis aspects. All together the patient was diagnosed with hemophagocytosis and was treated according to HLH protocol 2004, with improvement in skin pain symptoms, nodular lesions and dyspnea.However, a left superior limb force deficit was observed. The histological examination of skin biopsy performed earlier was completed: a diagnosis of non-Hodgkin's lymphoma Intravascular large B-cell (IVLBCL) was formulated. Patient developed fever and marked hypotension. A progressive failure of respiratory and cardiovascular clinical status and pancytopenia were observed. Chest X ray was consistent with a pulmonary infection. Aspergillus spp was isolated from bronchial aspirate colture.The patient died four months after appearance of first symptoms. *Conclusions:* IVLBCL is extremely heterogeneous in its clinical presentation and has been described in the small vessels of nearly every organ, leading to ischemia, organ dysfunction, and organ failure. Therefore, it has also been called the oncologist's "great imitator." In case of patients presenting with central nervous system and cutaneous involvement in presence of B symptoms this possible diagnosis might be taken into account.

PU026

IMAGING MRI IN DIAGNOSIS OF KIDNEY DLBCL-LYMPHOMA

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Introduction: Lymphoma is the most common blood cancer. Diffuse large B-cell lymphoma (DLBCL) is the most common form of non-Hodgkin lymphoma (NHL), accounting for about 30% of newly diagnosed cases of NHL in United States. The initial symptoms of DLBCL include painless swelling in one or more lymph nodes. Some people with DLBCL develop large tumors in the abdomen and kidney.We report a 65-year-old man with abdominal pain and history of chronic anemia. Abdominal MRI scan detected hyperintense lesions in the kidney and lomboaortic nodes >1cm.

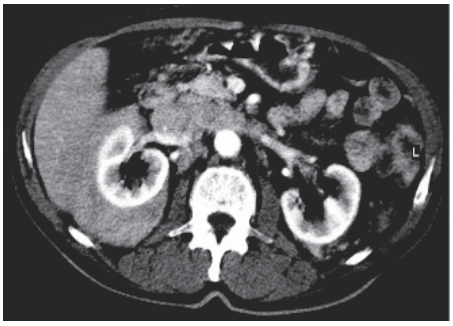

Figure 1. Hyperintense lesions and lomboaortic nodes >1cm in kidney diffuse large B-cell lymphoma (DLBCL).

Methods: A 65-year-old man with history of chronic anemia was admitted to our hospital for abdominal pain, light increase in serum creatinine activity and presence of hypoechoic lesions in the kidney. Abdominal MRI confirmed hyperintense lesions in the kidney and lomboaortic nodes >1cm. Bone marrow aspirate showed increased number of B lymphocytes 75%, with dysplastic features. Immunophenotype was CD20+, CD79+, BCL-2+, BCL-6 +, CD30-, CD15-, CD10-, CD3-. Histology of kidney lesions and abdominal nodes prompted the diagnosis of DBCL. *Results:* The patient was treated with R-CHOP (rituximab, cyclophosphamide,doxorubicin, vincristine and prednisone) obtaining a remission of symptoms. *Conclusions:* DLBCL is an aggressive (fast-growing) lymphoma that can arise in lymph nodes or outside of the lymphatic system, in the gastrointestinal tract or in the kidney. A possible strategy is to perform US as the initial technique in all patients with acute abdominal pain, with CT performed in all cases of nondiagnostic. US data on the use of MR imaging for this indication are still sparse. The use of conventional radiography has been surpassed; this examination has only a possible role in the setting of bowel obstruction.

PU027

PEGYLATED LIPOSOMAL DOXORUBICIN MAY INDUCE LONG-LASTING RESPONSE IN HHV8 RELATED KAPOSI'S SARCOMA POST IMATINIB TREATMENT FOR CML: A CASE REPORT

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Imatinib is the first inhibitor of BCR-ABL1 tyrosinase-kinase (TKI) able of inducing profound and long-lasting responses in most patients with chronic myeloid leukemia (CML). The long-term side effects of TKIs include secondary malignancies likely due to the capacity of the TKIs in impairing tumor immunity, preventing DNA damage repair and promoting genetic instability. Acquired immunodeficiencies, including AIDS and iatrogenic immunosuppression after prolonged treatment with corticosteroids and rituximab, as well as after organ transplantation, may be responsible for the development of Kaposi's Sarcoma (KS). Furthermore, human herpesvirus type 8 (HHV-8) has been shown to play a causative role in AIDS-associated KS as well as in primary effusion lymphoma and the plasma cell variant of multicentric Castleman's disease. We report a case of KS in a 70-year-old male with CML during imatinib treatment. This patient, in complete molecular remission (CMR), developed multiple violaceous nodular and plaque skin lesions on his left leg after 56 months of imatinib therapy (300 mg/day), followed after 2 months of imatinib discontinuation by supra- and subdiaphragmatic lymphadenopathies with increased fluorodeoxyglucose (FDG) uptake on positron emission tomography (PET) scans. A lymph node biopsy showed morphologic and immunohistochemical findings of a KS. In addition, the patient showed plasma levels of HHV-8 viremia of about 10.000 copies/mL, while serology and polymerase chain reaction (PCR) assay for HIV infection were negative. As the efficacy of recombinant interferon alpha (IFNa) for the treatment of KS and CML with approximately 30% of responses has been well documented in both diseases, we started IFNa (3 million units, 3 times a week) and continued it for 12 months, when was documented a partial response for KS, a simultaneous loss of CMR for the BCR-ABL, and a persistence of high levels of plasma HHV-8 DNA viremia (about 20000 copies/mL). Six months later the patient, for progression of KS, started treatment with pegylated liposomal doxorubicin (Caelix: 25mg/mq) every 2 weeks, associated with imatinib (200mg/day), achieving within six months a very good partial response for KS, documented by PET/CT imaging, and a new CMR for CML persistent up to now. In conclusion, pegylated liposomal doxorubicin at the doses employed can be safely administered and has shown efficacy in KS developed during long-lasting imatinib therapy for CML.

PU028

NON-HODGKIN LYMPHOMA WITH CARDIAC INVOLVEMENT

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Background: Primary cardiac lymphoma is an extremely rare occurrence, while secondary involvement of the heart during aggressive lymphoma can be quite common as autoptic finding. Diagnosis is often difficult because symptoms, including chest pain, dyspnoea and/or syn-

cope, are usually very subtle and non-specific; however improving imaging techniques can now help us detect such condition. *Description:* A 30-year old man with no history of cardiovascular events, was referred to our centre after the demonstration of a primary mediastinal large B cell lymphoma. FDG-PET scans at diagnosis did not show a direct cardiac involvement. Initial assessment including electrocardiogram, complete blood count, renal and liver function tests with LDH, was normal. A few days after admission the patient developed atrial fibrillation that was treated with IV amiodarone with restoration to sinus rhythm. Transthoracic echocardiogram showed asymmetric eccentric hypertrophy of left ventricle, diffuse hypertrophy and increased echogenicity of the right ventricle as per infiltration or deposition of amyloid, with preserved left ventricular function and mild pericardial effusion. Cardiac MRI confirmed thickened right ventricular walls with dyskinesia of the basal segment and interventricular septal involvement due to cellular infiltration. Late gadolinium enhanced images showed transmural pattern of medium-apical segments of the anterior and lateral walls of the right ventricle. Standard treatment of PMBL is rituximab based chemotherapy (i.e. CHOP); we decided to avoid daunorubicin for the first cycle, due to the risk of cardiotoxicity. After 3 weeks of treatment the transthoracic echocardiogram showed normalization of the myocardial wall thickness, disappearance of pericardial effusion and the patient did not experience any other rhythm alterations. *Discussion:* Transthoracic echocardiography is the first line imaging exam to be performed to evaluate heart function and can seldom demonstrate a direct cardiac involvement during lymphomas. Cardiac MRI is a very useful tool to describe a mass and its relationship with adjacent structures, and can also study an eventual myocardial infiltration. In our patient, in accordance with few data available in literature, we opted for systemic therapy comprising rituximab with prompt disappearance of myocardial infiltration, as demonstrated by echocardiography, and no events of cardiotoxicity related to therapy.

PU029

PRIMARY CARDIAC DIFFUSE LARGE B-CELL LYMPHOMA IN A ELDERLY PATIENT. A CASE REPORT

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Introduction: Primary Cardiac Lymphoma (PCL) is a rare extranodal lymphoma that involves only the heart and/or pericardium and is frequently diagnosed at autopsy. PCL is very uncommon in immunocompetent patients. *Methods:* A 87-year-old man, immunocompetent, developed heart failure fibrillation; echocardiography showed a large right atrial mass of probable tumoral origin. Imaging confirmation and staging included transesophageal echocardiography, CAT scan, MRI and PET. Blood count, LDH, virologic tests were normal. Bone marrow biopsy was not done. The heart tumor biopsy revealed a diffuse large EBV negative B-cell Lymphoma. Four weeks chemotherapy with cyclophosphamide, mitoxantrone, prednisone, vincristine, bleomicyn, etoposide was firstly performed and repeated with rituximab after three weeks. Drugs doses were slowly reduced. Bacterial and Pneumocystis carinii prophylaxis with cotrimoxazole was performed. G-CSF was administrated for 5 days. Follow-up evalutations were made by echocardiography and PET. After the first cycle of chemoterapy the patient developed Klebsiella Pneumonaie sepsi and liver toxicity. Drugs doses were further reduced. After three cycles of chemoterapy the disease was in partial remission, in complete remission at the end of treatment. *Conclusions:* Age is an important prognostic parameter, in patients with advanced high-grade non-Hodgkin's lymphoma (HG-NHL): these patients require more intensive and extensive therapy to obtain a good chance of cure. Patients with PCL have variable clinical manifestations that may lead to misdiagnosis and delay in treatment. PCL should be included in the differential diagnosis of a right atrial mass. Modern radiologic imaging allows earlier detection of these tumors. Aggressive therapy (VNCOP-B plus R and G-CSF regimen) can obtain long survival times.

PU030

RETROSPECTIVE EVALUATION ON EFFICACY AND FEASIBILITY OF R-CODOX-M/IVAC REGIMEN IN AGGRESSIVE DLBCL

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Diffuse Large B Cell Lymphoma (DLBCL) is an heterogeneous group of diseases whose behavior can be predicted by clinical risk scores, immunohistochemistry and cytogenetic. Among DLBCL, double hit lymphomas (DHL) and double or triple-protein-expression lymphomas (DPLs, TPLs) display a worse outcome and the standard frontline treatment with R-CHOP showed a poor outcome. From January 2011 in our centre (R-CODOX-M/IVAC regimen has been adopted as first line in aggressive DLBCL, defined by at least one among these features: high tumour burden, DPLs, IPI score >3 or by the presence of at least 1 extranodal site. Our aim was to define the efficacy and feasibility of this strategy and identify the subgroups of patients who may benefit from this approach. We retrospectively analyzed 20 patients affected by aggressive DLBCL treated with R-CODOX-M/IVAC. According to Ann Arbor classification, 11 patients were on stage IV, 1 on stage III, 3 in stage II and 5 in stage I. Median IPI score was 3. Eleven patients had DPLs and 4 of them had TPLs. After a median follow-up of 28 months, 5 patients died (25%), OS at six and twelve months was 89,4 and 70,4%, respectively, median not reached (NR). Complete remission was achieved in 11 patients (69%), partial remission in 2 patients (13%). The overall response rate was 82%. Three patients (18%) were primary refractory. Among DPLs, OS at six and twelve months was 88,9 and 64,8%, respectively, not significantly lower than non DPLs patients (p=n.s., median NR). In patients with Ann Arbour stage III or IV, OS at six and twelve months was 90,9 and 60,6% (median NR). In patients with IPI score >3, OS at six and twelve months was 78,8 and 45% (median 12 months). The main toxicity during CODOX-M was grade >2 mucositis (63%). Infections occurred in 71% of patients. Renal and liver toxicity was mainly of low grade. In IVAC regimen the main toxicity was haematological with 7 days of median duration of severe neutropenia (range 3-10), and 7 days (range 6-23) of thrombocytopenia. R-CODOX-M/IVAC is a generally well tolerated regimen, with acceptable toxicity profile for aggressive DLBCL. Our results suggest a potential benefit for DPLs, whereas higher IPI scores retains a negative prognostic impact. The next step will be retrospective FISH evaluation of C-MYC, BCL2 and BCL6 translocations, for lacking patients, in order to disclose a potential benefit for double or triple hit lymphomas.

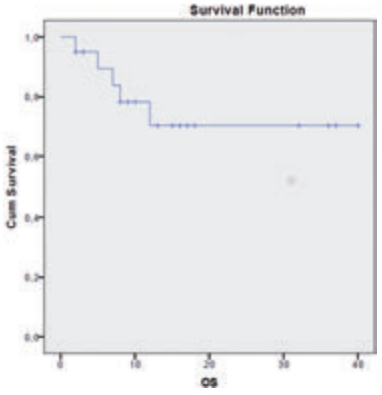

Figure 1.

PU031

ADULT T-CELL LEUKEMIA: NOVEL CHALLENGE FOR ITALIAN HEMATOLOGISTS

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Background: Adult T cell leukemia-lymphoma (ATL) is an aggressive lymphoid neoplasm that occurs in patients with human T-lymphotropic virus (HTLV-1) infection. Shimoyama described four clinical subtypes distinguished by clinical features and prognosis and among these acute ATL is associated with the poorest prognosis. Japanese trials

containing polychemotherapy improve response rate, without affecting survival, while the combination of interferon- (IFN-) and Zidovudine (AZT) has been reported to favourable impact on both. However, patients carrying a mutated p53 fail to respond also to this therapy, requiring only ablative treatment. *Aims:* We described a case of HTLV-1-related ATL refractory to combination of AZT and IFN-. *Results:* A Gambia-born 32-year-old man was admitted at our hospital with hyperleucocytosis and acute kidney failure with hypercalcemia. The lymphocytes phenotype evaluation performed on peripheral blood (PB) and bone marrow (BM) samples revealed the expressions of CD3+ CD5+ CD25+ CD4+ CD7-, suggestive of ATL. Typical ATL "flower cells" were observed on the peripheral blood smear. Serological research for HTLV-1 confirmed the infection. Total body CT scan revealed multiple enlarged lymph nodes and lung involvement. According to a recent meta-analysis, we started AZT 750 mg/day and dose escalated IFN- 5 million IU/m^2/day SC. Antiviral and fungal prophylaxis were started. After two weeks we observed a significant reduction of lymphocytes. These results lasted only one month shortly followed by disease reappearance (pathological lymphocytes). The serological research for HTLV-1 detected high rate of replication (1/5000 cells). Therefore it was decided to start chemotherapy (CHT) according to mLSG-15 regimen: VCAP (vincristine, cyclophosphamide, doxorubicine, prednisone), ACP (doxorubicin, carmustine, prednisone) and VECP (vinorelbine, etoposide, carboplatin, prednisone), for a planned number of six cycles (cy). At this moment, following the first cy, a prompt lymphocytes response has been observed and the patient is undergoing subsequent cy of chemotherapy with on going uunrelated donor search for allogenic stem cells transplant. *Conclusions:* Adult T-cell leukemia is an aggressive malignancy rarely observed in the European countries. The limited literature on this rare disease supports the combination of AZT and IFN-, while in our experience CHT is needed in order to plan in young patients allogeneic transplantation procedures.

PU032

NOT ONLY NON HODGKIN LYMPHOMA

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We describe a clinical case of a man 73 years old, arriving in hospital because fever. He had a history of 3 years mild lymphocytosis and suspect lymphoproliferative disease at peripheral immunophenotype. At physical examen he had also splenomegaly and cervical lymphoadenopathies, mild thrombocytopenia and anemia, leucopenia with only slight alteration of flogosis test. Also CT scan not showed foci of infections, but confirmed thoracic and abdominal lymphoadenopathies (max 2cm) and splenomegaly. Patient had fever despite of empiric antibiotic therapy, and a progressive pancytopenia. A bone marrow hystology confirmed clinical suspicion of lymphoma, but only a 30 percent of lymphocytes and also a normal number of megakaryocytes and erithroid precursors. Fever responded only to steroid, but not pancytopenia. Also steroid 1mg/kg and igvena not improved pancytopenia, and appeared also signs of liver deficiency, with a huge increase of ferritin. At the cytology revision of bone marrow we founded some pictures of phagocytosis of blood red cells, platelets and neutophils. In the suspicion of lymphohistiocytosis we repeated a bone marrow cytology and performed a dosage of soluble cd25 receptor. These last tests was diagnostic for lymphohistiocytosis associated to non hodgkin lymphoma marginal type. Pet test was intensely positive at lymphonodes, in spleen and marrow we thinked related to lymphohistiocytosis more than lymphoma. We start then protocol including desametazon, etoposide (with dose reduction related to age of our patient, with weekly infusion HLH 94) and weekly infusion of rituximab. He also received antibacterial prophylaxis with fluconazol, fluorochinolonic, and pentacarinat aerosol for pneumocisti jiroveci. Only at third week we administered a filgastrim two days therapy. After 4 week of treatment we registered a amelioration of clinical status, a surprising normalization of blood count, a normalization of liver blood tests and a reduction of cd25 receptor, that was almost normal. At our knowledge this is the first report of lymphohistiocytosis associated to low grade non hodgkin lymphoma and is a interesting case of clinical reasoning leading to research of rare etiology capable to justify all clinical data.

PU033

REVISED LENALIDOMIDE DOSING FOR MYELOMA PATIENTS RECEIVING DIALYSIS: SAFETY AND SUCCESSFUL

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Introduction Relapsed/refractory Multiple Myeloma (rrMM) requiring hemodialysis is a life-threatening condition and treatment options are limited. Since lenalidomide (L) is primarily excreted unchanged by the kidney, adjustments to the starting dose are recommended. Unfortunately, there are limited experiences in patients with renal impairment who received L, and severe adverse events occur with a high frequency in patients with rrMM requiring hemodialysis treated with L. Discussions We reported our retrospective study of four patients (4 male; median age 73.5 years [range 62-77]) with rrMM requiring dialysis before receiving "lenalidomide plus desamethasone" (LD). Three patients developed renal failure before MM diagnosis, one patient during progressive disease development. A different L dose adjustment (5 mg every other day on days 1-21 of a 28-day cycle after dialysis) from the recommendations of the manufacturer was chosen due to additional fragilities (serious comorbidities and advanced age). Until initiation of therapy with L, renal failure persisted for a median of 28 months (range 26-96).All patients achieved at least a PR. No patients with partial or complete myeloma remission received partial or complete renal response.

Table 1. Characteristic Patients.

Median (range) Age, y	73.5 (62-77)
ANC 10^3/µL (range)	2.9 (2.8 – 3.3)
Hgb g/dL (range)	12 (9,3 – 12,6)
Platelet 10^3/µL (range)	124 (59 – 225)
Creatinine Clearence ml/min (range)	not required
Comorbidity	
• Diabete Mellitus	yes
• Hypertension controlled by medication	yes
• Atrial Fibrillation or other cardiac arrhythmias	yes
• Chronic obstructive bronchopneumonia	yes
• Reversible ischemic attacks	yes
• Vascular cerebropathy	yes
median number of prior therapies	2 (1-2)
median number of lenalidomide cycles	12 (6-19 cycles)
ORR with previous treatments (n. patients)	4
• PR	3
• VGPR	1
• CR	0
ORR with "lenalidomide + dexamethasone" (n. patients)	4
• PR	2
• VGPR	1
• CR	1
median duration of response, months (range)	13,5 (6-21)
Non-Hematological Toxicities (grade ≥ 3)	0
• Diarrhea	0
• Constipation	0
• Thromboembolic events	0
• Infections	0
Hematological Toxicities (grade ≥ 3)	
• Neutropenia	0
• Thrombocytopenia	1

The median number of L cycles was 12 (range 6-19 cycles). The median time to best response was 3,5 months (range 2-6). After 24 months, all patients are alive at the time of analysis and at time of last follow-up; 2 patients were still receiving "LD", preserving haematological response reached; 2 patients stopped treatment due to progression, with a median duration of response of 13,5 months (range 6-21). Only one patient developed grade 3 thrombocytopenia without blending events. No grade 3-4 neutropenia, gastrointestinal adverse events, thromboembolic complications or serious infectious episodes were reported. Conclusion Different L dose adjustment (5 mg every other day on days 1-21) from the recommendations reported in the approved summary of product characteristics is associated with a high response rate and a long duration of response. In addition, we observed a lower-than-expected incidence of haematological toxicities without an excess of prolonged cytopenia despite long-term exposure to L. L dose adjustments minimize excessive toxicities and make the combination "LD" as an effective and well tolerated therapeutic option also in very frail/unfit patients with rrMM requiring dialysis.

PU034

SYMPTOMATIC MULTIPLE MYELOMA COMPLICATED BY HEPATITIS C VIRUS REACTIVATION: SIMULTANEOUS ADMINISTRATION OF NEW ANTIVIRAL DRUGS AND CHEMOTHERAPY

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Introduction: HCV-infected cancer patients are often excluded from receiving conventional or aggressive cancer therapy and participating in clinical trials because of "HCV reactivation" (elevation of viral load [HCV-RNA] by ≥1 log10 IU/mL from baseline), a more rapid development of cirrhosis and poor survival rates. A new group of direct acting antiviral agents (DAA) have dramatically improved rates of sustained virological response and have made chronic HCV infection curable. *Patients and Methods:* Patient 1 A 79-year-old woman with a 30-year history of genotype 1b chronic HCV infection was diagnosed with symptomatic IgGk MM in June 2015. Treatment with bortezomib (1.3mg/m^2 on days 1, 8, 15, 22) plus dexamethasone (20 mg on the days 1-2, 8-9, 15-16, 22-23) was given. Patient 2 A 66-year-old woman was diagnosed with symptomatic IgGk MM and genotype 1b chronic HCV infection in May 2015. Treatment with bortezomib (1.3mg/m^2 days 1, 4, 8, 11, 22, 25, 29, and 32, cycles 1 to 2; days 1, 8, 22, and 29, cycles 3 to 9) plus melphalan (9mg/m^2 days 1 to 4, all cycles) and prednisone (60mg/m^2 days 1 to 4, all cycles) was instituted.

Table 1. Benefit of direct-acting antiviral treatment in all patients.

	Simultaneous administration of chemotherapy and DAAs								
	Patient 1			Patient 2			Patient 3		
	before CHT	during CHT	after DAAs	before CHT	during CHT	after DAAs	before CHT	during CHT	after DAAs
ALT (IU/mL)	35	175	14	34	136	32	25	79	17
HCV-RNA (IU/mL)	903.000	40.400.000	undetectable	297.000	677.000	undetectable	371.000	2.070.000	undetectable

Patient 3 A 77-year-old woman with history of genotype 2a/2c chronic HCV infection was diagnosed with symptomatic IgGk MM in November 2014. Treatment with bortezomib (1.3mg/m^2 on days 1, 8, 15, 22) plus metilprednisone (60mg/m^2 on days 1-4) and melphalan (9mg/m^2 on days 1-4) was given. Four months after the end of the treatment, lenalidomide (15mg) plus dexamethasone (20 mg as a once-weekly administration), due to progression disease, was instituted. The dosage of lenalidomide was modified according to the patient's clinical features. After a median time of 270 days (range 235-570) from the start of chemotherapy, HCV reactivation was noted. DAA therapy with sofosbuvir-containing regimens was given. Within 12-24 weeks of treatment with DAAs, serum ALT levels normalized, and HCV-RNA became undetectable, showing that HCV infection is curable also in cancer patients. The choice of DAAs regimen was guided by the specialist on infectious diseases. *Conclusions:* Simultaneous administration of chemotherapy and DAAs should be used with extreme caution because of possible drug-drug interactions. In our experience, the concomitant administration of DAAs and chemotherapy guaranteed the eradication of HCV and provided an appropriate anti-cancer therapy exposure without discontinuation.

PU035

IGM MGUS (IGM MGUS) EVOLVING IN IGM MULTIPLE MYELOMA (IGM MM): A CASE REPORT WITH BLEEDING ONSET

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Introduction: IgM MM is a rare immunoproliferative disease(<0.5% of all myelomas) associated with an aggressive clinical course and poor prognosis. The diagnosis is very difficult, and is crucial to distinguish between Waldenstrom's macroglobulinemia (WM) and IgM MM because the prognosis and treatment strategies are different. Materials and methods: on January 2017 a 63 years old women, after 10 years MGUS, was admitted in our ward for frequent bowel bleeding and skin lesions like-erysipelas. Our first hypothesis based on clinical features and the presence of bleeding associated to IgM protein was WM. Diagnostic tools were planned. Physical and ultrasound examination excluded oragomegaly or lymphoadenopathy. Gastroscopy was negative, colonscopy showed sigma diverticula. Laboratory finding were: Hb 9g/dL, WBC 3.7x109/L, platelets 100x109/L; creatinine 0.98mg/dL, calcium 10.30mg/dL, LDH 253 U/L; active partial thromboplastin time 54 sec. Electrophoresis revealed a homogeneous spike in the gamma region identified as an IgM, the level was 7120mg/dL, with a low level of IgG and normal of IgA. Infectious and virological work up was negative. Cryoglobulins was absent. Bone marrow biopsy revealed a diffuse infiltration with 70% atypical plasma cells positive for CD38-CD138, CD19, CD56, CD45-were negative. Cytogenetic showed t(11;14) abnormality. MRI of spine and skull and pelvis revealed some lytic bone lesions. Diagnosis of symptomatic IgM MM was made, based on clinical and laboratory evidence; so the patient started a combination therapy with bortezomib and dexamethasone.Results:in our case the hyperviscosity, related to the absolute level and structure of the IgM pentamer, caused the bleeding events at onset. After the second cycle, IgM value is gradually reduced (5400 *vs* 7120), bleeding was stopped. We have planned 4/6 cycles. Conclusion:IgM MM requires a tailored treatment and supportive therapies to prevent complications. In case of IgM paraproteinaemia, without organomegaly and lymphadenophaty the suspicion might been on IgM MM. A correct diagnosis must be based on the bone marrow trephine, flow cytometric immunophenotyping and radiology imaging (MRI). Regarding the differential diagnosis, the presence of multiple lytic bone lesions with bone marrow plasma cell infiltration supports the diagnosis of MM. A strict definition of the entity, frequent follow-up are needed to control the IgM value eventually make modification of the treatment plan.

PU036

AN UNUSUAL AMYLOID DEPOSITION: AMYLOID ARTHROPATHY. A CASE REPORT

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Amyloid light-chain (AL) amyloidosis is a rare plasma-cell dyscrasia caused by tissue deposition of amyloidogenic light chains in the form of abnormal, insoluble fibres. A 57yo woman of Nigerian heritage was diagnosed with monoclonal gammopathy of uncertain significance on a regular check-up in 2012. She was followed up every 6 months with clinical evaluation and laboratory tests. Three years later (Feb 2015) the patient developed severe joint pain exacerbated during normal daily activities. PET/CT revealed 18F-fluorodeoxyglucose focal uptake in the right scapula with a maximal standardized uptake value (SUV) of 6,4, 8th right rib (SUV 6,1), L1 vertebral level (SUV 7,3) and multifocal uptakes in the kiss (maximum SUV 9,3). BM appeared extensively (80%) infiltrated by CD138+ plasma cells. A diagnosis of Durie-Salmon stage/ISS stage II multiple myeloma (MM) was made. She underwent induction treatment with 4 cycles of the triplet combination of bortezomib, thalidomide, dexamethasone (VTD). The pt achieved partial response. In Jan 2016 she was admitted in our hospital for autologus stem cell transplant (ASCT). Physical examination revealed multiple painful joint deformity, edema and rigidity involving knees, ankles, wrists and

small joints of the hands (especially right hand) and feet. She also showed ulnar devition of her fingers and prehension deficit. Periumbilical fat biopsy was positive for amiloid deposition at the electronic microscopy. The study of the right hand X-ray revealed signs of arthrosis (in accordance with amyloid arthropthy) that involved distal interphalangeal joints of the 2nd and 3rd finger and proximal interphalangeal joint of the 2nd finger. She received conditioning regimen (melphalan 140 mg/sqm) followed by ASCT (19th of Jan). At the last follow-up, February 2017, the patient was alive and in complete hematological response. The extension of amyloid arthropathy was reduced with consequent improving of normal physical activities. AL deposition associated with MM may lead to an arthropathy resembling rheumatoid arthritis (RA). A literature review made by Ahmed M. Elsaman *et al.* found only 101 reported pts with amyloid arthropathy between 1931 and 2012. Pts often manifested joint involvement before the diagnosis of MM and about 1/3 of them were misdiagnosed as RA. Our case highlights the importance of prompt recognition and specific treatment of this disorder as a critical point to reduce morbidity and mortality.

PU037

SAFETY AND EFFICACY OF SUBCUTANEOUS VERSUS INTRAVENOUS BORTEZOMIB IN PATIENTS WITH NEWLY ELDERLY DIAGNOSED MULTIPLE MYELOMA

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Background: Bortezomib in association with melphalan ad prednisone (VMP) is approved for newly diagnosed multiple myeloma (MM) not eligible to transplant. Intravenous injection was the standard route of administration but few studies described subcutaneous (SC) injection safe and efficacy. *Methods:* we described 80 newly diagnosed MM, collected retrospectively, treated upfront with VMP with bortezomib given SC or IV, to compare efficacy and safety of the different routes of administration. VMP regimen was 5 weekly-cycle, bortezomib 1.3 mg/mq, days 1,8,15,22; Melphalan 9 mg/mq days 1 to 4; Prednisone 60 mg/mq, days 1 to 4 for a maximum of 9 cycles. *Results:* From January 2010 to November 2013 36 pts (45%) received IV bortezomib, whereas from July 2012 to June 2016 44 pts (57%) received bortezomib SC (Table 1) Among patients treated with bortezomib IV, 5 (16%) discontinued treatment within 4 cycles due to toxicity or no compliance, 6 (18%) stopped therapy after 6 cycles mainly for neurological toxicity grade >2, 25 (69%) patients completed 9 cycles. In the group of SC administration therapy was interrupted in 3 pts for disease progression and in 6 (13%) cases for toxicities whereas the other 35 patients completed 6 cycles or 9 cycles. Overall response rate (>PR) was evaluated only in patients that completed 9 cycles of therapy and was 75% in IV administration and 82% in SC, and very good partial response rate or better was 52% and 51%, respectively. Adverse events (grade 1 to 4) were reported in 32/36 (89%) pts treated with bortezomib IV and in 38/44 (86%) in the SC group. Hematological toxicity (grade >3) included neutropenia (19%), anemia (6%) and thrombocytopenia (25%) in patients treated with bortezomib IV, whereas in those treated with SC neutropenia was referred in 22%, anemia in 9% and thrombocytopenia in 22%. Non hematological AEs of grade >3 mainly included infections (9% in IV *vs* 6% in SC), gastrointestinal toxicity (9% *vs* 4%) and peripheral neuropathy (9% *vs* 4%). *Conclusions:* Different bortezomib administration demonstrated no substantial difference in term of response rate. Hematological toxicity was similar in the two groups, however non-hematological toxicity was higher in IV group, particularly for infections and neuropathy.

Table 1.

Patients characteristics	IV	SC
Age, median (range)	71 (59-82)	74 (67-84)
M/F	16M/20F	21 M/23 F
ISS stage	Stage I: 17 stage II: 8 stage III: 11	stage ISS I: 21 stage II: 12 stage III: 11
IgG,IgA;IgD	22; 8;0	27;11;1
FLC; Non-secretory	3;3	4;1

PU038

RUXOLITINIB IN PRIMARY MYELOFIBROSIS ASSOCIATED WITH SPLANCHNIC VEIN THROMBOSIS COMPLICATED BY GASTROINTESTINAL BLEEDING: A CASE REPORT

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Myeloproliferative neoplasms (MPNs) are hematologic disorders characterized by clonal proliferation of myeloid lineage cells. Arterial and venous thrombosis are one of the major causes of morbidity in Ph negative MPNs. Venous thrombosis may occur at unusual sites such as splanchnic vein thrombosis (SVT). The presence of JAK2 V617F mutation increases the risk of SVT. Moreover there is a high incidence of gastrointestinal bleeding in patients with SVT. We present a case of a 63 years old woman with a history of JAK2-unmutated myelofibrosis, treated with ruxolitinib for two months, recently splenectomised for a trauma, who went to emergency room with abdominal pain. Laboratory test evidenced normal liver and renal function, leukocytosis ($29x10^9$/L) and thrombocytosis ($1680x10^9$/L). A computed tomography (CT) with intravenous contrast showed portal and residual splenic vein thrombosis despite heparin prophylaxis. The patient was started on anticoagulation with low-molecular-weight heparin (LMWH) at therapeutic dose and cytoreductive therapy with hydroxyurea. After three days of hospitalization, the patient presented a massive lower gastrointestinal bleeding resulting in severe anemia. Anticoagulant therapy was discontinued and transfusion supported care was started. Endoscopy revealed hemorrhagic rectal ulcers. Acute bleeding was successfully controlled with endoscopic hemostasis. After the complete resolution of acute bleeding, confirmed by endoscopy, anticoagulation was restarted. After three months, echo-doppler analysis evidenced resolution of thrombosis and a cavernous transformation of the portal vein with multiple collateral veins. Then, the patient began ruxolitinib at the dose of 10 mg BID. After 12 weeks of treatment, no hemorrhagic adverse events were recorded and splanchnic circulation status was unchanged. No hematological toxicities were observed. MPN-related symptoms improved significantly. This case shows that ruxolitinib is safe in patients with MPN-associated SVT with hemorrhagic complication.

PU039

MOLECULAR STUDY OF CALR GENE: MYELOPROLIFERATIVE NEOPLASMS (MPN) WITH NEGATIVE PHILADELPHIA AND JAK2/V617F

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In the last years there has been a improved interest in the molecular characterization and classification of myeloproliferative neoplasm (MPN) and target therapy. Mutation in Calreticulina (CALR) could be considered a new markers in diagnosis of MPN, have been found in most patients with essential thrombocytemia (ET) and idiopathic myelofibrosis (MF). It's important to underline that, at patients with negative JAK 2 V617 mutations, are often associated mutations in the CARL and MPL gene, in particular in the receptor binding region. Therefore, detecting the both mutations in patients JAK2 negative can be allow a correct diagnosis of MPN. In this study, we plan to analyze CALR and MPL mutations in suspect myeloproliferative neoplasm Ph-negative and JAK2 negative. A second purpose is to identify a possible association between type of mutations, (type 1 or type 2) for CALR, and allelic status for MPL positive patients and the risk of hematologic malignancy progression. We have been enrolled 98 patients, 22 cases with the BCR-ABL rearrangement (22.4%)were diagnosed as CML and not included in this evaluation. Seventy six patients: 32 men and 44 women (age: 5-82; average: 53,43; median: 58,0) afferent to the Hematology Department of Cancer Hospital for diagnostic hypothesis of MPN. From peripheral blood samples, DNA and RNA extracted were used for molecular studies. BCR/ABL transcript and JAK2 mutation analysis had already been done for all samples, by qualitative PCR and quantitative PCR with allelic discrimination. Cases involved in the present study were been analyzed for W515 L/K MPL mutation using a commercial kit and for CALR with homemade kit. Only samples negative for JAK-2 mutation were subjected to CALR and MPL mutation

screening. Among 76 cases of BCR-ABL negative of MPN patients, 21 cases had (29.57%) JAK2 V617F mutation, 4 case(5.26%)CALR mutation type 1 and two cases (2.6%) W515 L MPL mutation. The CALR gene screening, in qualitative PCR is rapid, sensitive and can be show all insertions and deletions of CALR, later confirmed with sequencing analysis. The quantitative allelic discrimination evaluation in MPL (ratio FAM/VIC) could be used in the monitoring of minimal residual disease (MRD). The proliferative increase of bone marrow, complications and risk of thrombotic and embolic occurrence of events are lower in CALR mutated patients than those in JAK2 V617F mutated patients. No association between type and disease progression.

PU040

CLINICAL UTILITY OF THE SERUM FREE LIGHT CHAINS (FLCS) KAPPA AND LAMBDA ASSAY IN THE FOLLOW-UP OF PATIENTS WITH MULTIPLE MYELOMA (MM) UNDERGOING AUTOLOGOUS PERIPHERAL BLOOD STEM CELLS TRANSPLANTATION (PBSCT)

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FLCs assay is an important marker for diagnosing and monitoring transplanted patients with multiple myeloma (MM). It is a very sensitive test that allows the early detection of relapse especially when serum protein electrophoresis (SPE) fail to yield a response due to the absence or low level of the monoclonal component (CM). The measurement of k/ ratio precociously indicates the response giving clinicians the possibility to change the treatment at the right time. In this study we described the evolution of FLCs levels and k/ ratio *vs* CM in 7 patients with MM undergoing autologous stem cells transplatation (PBSCT). All parameters were analyzed during the following times: onset of the disease, before transplantation and after transplantation with monitoring every 3 months in order to evaluate the k/ ratio as predictive marker of relapse. One patient showed a very good partial response (VGPR) until 9 months post PBSCT. After this period the k value greatly increased even though the CM value remained unchanged, suggesting the disease progression as confirmed later by observation aspirate and biopsy procedures. In this clinical case the k/ ratio was an early marker of relapse. In another clinical case we observed an extramidollary relapse as confirmed by Positron Emission Tomography (PET) and the only altered parameter was the k/ ratio increase. The analysis of clinical cases reported in this study has highlighted the extreme usefulness of monitoring serum concentrations of FLC for the disease progression and we can conclude that the ratio k/ could be considered an essential non-invasive early marker for multiple myeloma relapse in the follow-up of patients with MM undergoing PBSCT.

PU041

IMMATURE RETICULOCYTE COUNTS (IRF) IN ADVIA 2120: EARLY INDEX OF ENGRAFTMENT FOLLOWING ALLOGENIC TRANSPLANT IN LAM-HR PATIENT?

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Engraftment is an important critical point in allogenic stem cell transplantation. The IRF-H and IRF-M are new parameters in reticulocyte total component IRF. The IRF-H and IRF-M detected by ADVIA 2120 counter (SIEMENS); the value show the real status of different reticulocytes components. The IRF-H and IRF-M index, can be early indicator of engraftment in erythroid fractions. Generally, for engraftment evaluation, neutrophil and platelet values were indicate as the first days with neutrophil count >0.5x10(3)uL, and platelet count >20x10(3) uL. The immature reticulocyte fraction total (IRF) cutoff used was 0.5%. Follow- up in allo tranplantation patient with myloid acute leukemia in High Risk was studied, after 6.17 CD34/Kg donor infusion. Total, Immature reticolocytes (retic H and M) and total reticulocyte were evaluated in peripheral blood and measured by automatic counter ADVIA 2120. We have used blood, considered neutrophil and platelet values and new reticolocytes parameter as IRF-H and IRF-M, reticulocytes index % with low and mediun level of RNA, from 27 days by allotransplantation. After 12 days by allotransplant and for the next days absolute neutrophil count were 0.5x10(3) uL and platelet count 20x10(3)uL this evidence engraftment. The evaluation of reticolocytes % index, show IRF >0.5% at 5 days from allotransplant. The IRF-H single evaluation and IRF-H plus IRF-M new parameters in reticulocyte total components can be used as earlier indicator of engraftment after allotransplant. Increase of IRF H fraction and IRF-H plus IRF-M was observed 5 days before of increase of neutrophil value and platelet count, and this suggest an more early index respect neutrophil count and platelet count in engraftment post Allogenic stem cell transplantation.

PU042

DNA POLYMERASE CHAIN REACTION FOR PLASMA DETECTION OF CYTOMEGALOVIRUS REACTIVATION AFTER AUTOLOGOUS AND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Cytomegalovirus (CMV) is a common viral infection associated with significant morbidity and mortality after autologous and allogeneic hematopoietic stem cell transplantation (Auto-HSCT and Allo-HSCT). We evaluated for CMV reactivation 123 consecutive patients who underwent Auto-HSCT (85) or Allo-HSCT (38). Our cohort included 55 multiple myelomas, 27 acute myeloid leukemias, 19 non Hodkin lymphomas, 12 Hodkin lymphomas, 6 acute lymphoblastic leukemias, 3 myelodysplastic syndromes and 1 Ewing sarcoma, with a median age of 49 years (range 18-75) and a female to male ratio of 0.70 (male/female: 72/51). CMV monitoring was performed on plasma samples by a real-time TaqMan CMV-DNA polymerase chain reaction (PCR) assay, according to the manufacturers' recommendations (Roche). Quantitative CMV DNA for Auto-HSCT was detected patients once a week during hospitalization, then once a month for 6 months for, whereas we detected for Allo-HSCT patients CMV DNA twice a week for 100 days post-engraftment, and then once a month for the next 12 months. CMV reactivation was defined by plasma CMV DNA levels higher than 137 IU/ml in a single plasma sample. CMV reactivation was detected in 13 Auto-HSCT (15%) and 9 Allo-HSCT (24%), with a median CMV peak viral load of 650 UI/ml (range 169-92000) after a mean time of 32 days (range 5-60) for Auto-HSCT, and 1360 UI/ml (range 451-5510) after a mean time of 80 days (range 9->100) for Allo-HSCT. Preemptive therapy with oral Valgancyclovir (VGCV), at a dose of 450 mg twice daily, in 9 Auto-HSCT determined a prompt clearance of CMV viremia after a median time of 27 days (range 7-45), and in 8 Allo-HSCT, previously already treated with low dose VGCV as CMV prophylaxis, after a median time of and 20 days (range 7-56). 1 Auto-HSCT was treated with VGCV 900 mg x 2/die, and 3 patients, with clear evidence of haematological recovery, did not receive any drug, but were closely monitored with PCR after Auto-HSCT. Lethal CMV infections were documented in 1 Auto ad 1 Allo-HSCT. Our data suggest that plasma CMV DNA PCR real-time quantification is highly sensitive for identification of CMV reactivation, and its use in monitoring patients after HSCT can allow to quickly plan the best anti-CMV therapy.

PU043

HEMATOPOIETIC STEM CELL TRANSPLANT-ASSOCIATED THROMBOTIC MICROANGIOPATHY (TA-TMA) HAS HIGH-RATE MORTALITY WHEN TREATED WITH CONVENTIONAL APPROACHES

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Hematopoietic stem cell transplant-associated thrombotic microangiopathy (TA-TMA) is a fatal, multifactorial complication in allogeneic stem-cell transplantation, which occurs in 10% to 20% of patients, with unknown pathogenesis and multiple risk factors. We retrospectively investigated the incidence of this condition in 229 patients (58,9% male, median age 48,5 years) undergone allogeneic stem cell transplantation

at our Center from 1998. Fifteen patients (6,5%) developed TA-TMA (15,8% of deaths for transplant-related causes). The majority of patients with TA-TMAs (66.7%) were female, with a median age lower than patients who did not develop the syndrome (40,4 years). The incidence of TA-TMA was higher in lymphoid leukemias (13,2%) and in non-Hodgkin lymphomas (9,5%). Thirteen patients had a mismatched donor (11 MUD, 2 haplo). The median time of onset of TA-TMA was 11 days after transplant. Thirteen patients received fludarabine in conditioning regimen; at the moment of the onset of TA-TMA, all patients were in course of treatment with cyclosporine, 4 in association with mycophenolate mofetil. Mean clinical features at diagnosis were: schistocytes 4,7%; serum lactate dehydrogenase 1435 U/L; serum creatinine 2,6mg/dL; serum total bilirubin 4,3mg/dL; hemoglobin 8,5g/dL; platelet count 16400/mcL. The coagulation studies were normal. Pre-transplant median ferritin level was normal in these patients (226ng/ml), although they received a median of 5,5 red blood cells transfusions before the onset of TA-TMA, and a median of 3 platelets transfusions. Two patients had received granulocyte transfusions before transplant, and four received plasma infusions. Stem cell transplantation were administered after more than one chemotherapy line in 7 patients; the median of courses of chemotherapies in previous treatment was 6. Cyclosporine was immediately suspended in all patients when TA-TMA was suspected. All patients died due to TA-TMA progression despite plasma-exchange. Two patients were treated with defibrotide without any benefit. In conclusion, TA-TMA is a fatal condition, in which the therapeutic approach is still empiric and largely ineffective. An early diagnosis could be the key of the success of the treatment, together with an early discontinuation of calcineurin inhibitors. The role of plasma exchange is limited, but recent reports have indicated a benefit with the use of monoclonal antibodies (Rituximab and Eculizumab).

PU044

NOVEL IMMUNOMODULATING AGENTS AND MONOCLONAL ANTIBODIES IN MULTIPLE MYELOMA PATIENTS RELAPSED AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Allogenic stem cell transplantation (HSCT) can be a salvage option for selected young multiple myeloma (MM) patients with high-risk relapse; however, disease recurrence is a frequent event and a treatment with new immunomodulating agents (Imids) or monoclonal antibodies can potentially enhance graft-versus-myeloma effect or increase the risk of GVHD. Sixteen consecutive MM patients with first (9) or subsequent relapse (7) underwent HSCT in our Centre from January 2012 to December 2016. Median age was 49 years (range 33-62), 7 patients received fludarabine-thiotepa-busulfan and 9 fludarabine-melphalan conditioning regimen. Stem cells came from HLA-identical sibling donor in 3 cases and in the other 13 from 7 or 8/8 HLA matched unrelated donors. Thirteen patients (81%) were at least in PR at HSCT. A consolidation treatment with bortezomib twice a month was administered to patients with persistence of serum /urine monoclonal component at day + 100. At a median follow-up of 13,5 months after HSCT, 3 patients (19 %) died due to TRM, 6 pts (37%) are in continuous CR and 7 pts (44%) had clinical relapse. Median time between HSCT and progression was 5 months (range 3-34). The following treatments were administered: bortezomib, lenalidomide, desametasone (VRD) (1), pomalidomide (3), daratumumab (2), thalidomide (3) and donor lymphocyte infusions (DLI) (3). Median number of cycles administered was 3 (range 1-9). Reasons for stopping salvage treatment were: progression (4), severe infections (1 CMV corioretinitis) and GVHD (2). Both patients developing acute GVHD had received DLI after pomalidomide and daratumumab, respectively. At a median follow-up of 6 months from salvage initiation, out of the 7 patients relapsed after HSCT, 3 patients progressed and died, 1patient died of acute GVHD, 2 patients had stable disease and 2 patients are in continuous VGPR after daratumumab and pomalidomide, respectively. New Imids and monoclonal antibodies are feasible and promising treatments in MM relapsing after HSCT; however, the risk of severe infections and GVHD has to be taken in account, particularly in patients receiving DLI along with new drugs.

PU045

UNMANIPULATED HAPLOIDENTICAL-RELATED DONOR HCT: A SINGLE CENTER EXPERIENCE

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Introduction: In recent years, there is a remarkable trend in the use of haploidentical-related hematopoietic cell transplantations in patients who do not have a HLA matched related or unrelated donor. Here, we report our single-center experience, in patients who underwent haplo-HCT for acute leukemia. *Material and Methods:* Between 2011 and 2016, 17 consecutive adult pts, 7 males and 10 females, median age 42 years(range18-61years) with high-risk AML underwent unmanipulated, BM or PBSC transplantation from an haploidentical family donor. 11 pts transplanted for AML(5 in CR1, 1 in CR2, 1 in minimal active disease after CR1, 1 second trasplant in CR2, 1 tMDS in CR1, 2 AML secondary to IMF in CR1), 5 for ALL(2 in CR1, 3 in active disease) and 1 mastcell leukemia (secondary to AML) in active disease.16 pts received myeloablative conditioning, and 1 reduced intensity, respectively. In 5 pts stem cells source was BM, in 12 were G-CSF mobilized PBSC. The median infused CD34+ cell dose was 4.47x106 (range 1.0x106-8.2x106). Conditioning regimens were: BU-FLU-MAC(N=9), TBF-MAC (N=7), TBF-RIC(N=1). The regimens for GVHD prophylaxis were: PTCy as sole GVHD prophylaxis(N=1),MTX-CSA-ATG(N= 9),MP-ATG-Tacrolimus(N=6), ATG-CSA-MTX-MMF(N=1). *Results:* Sustained trilineage engraftment occurred in 15 pts(88%), 2 pts died of transplantation-related complications before day 21 after transplantation without myeloid recovery. For pts receiving BM or PBSC grafts, the median time to >500 neutrophils recovery was 16 days(range 10-36), and >20000 platelets recovery was 16 days(range 13-37). 7/15 patients (46.6%) and 2/15(13.3%) had II-IV and III-IV grade of acute GVHD, respectively.The incidence of grade II-IV cGvHD was 27%. After a median follow-up of 11 months, 4/17 pts(23.5%), 4 out 5 pts transplanted in CR1, are alive and disease free at 40, 28, 19, 16 months (inclusing the patient transplanted for AML after IMF). The 2-year probability of OS and PFS was 40%(95% CI, 4.0-58.0%) and 33.6%(95% CI, 2.0-38.0%), respectively. Causes of death were: sepsis(N=1), fatal aGvHD(N=1), pneumonia (N=1), toxicity(N=2), progression(N=4) and relapse(N=4). *Conclusions:* In our experience unmanipulated BM or PBSC transplantation from haploidentical family donor is feasible approach with high engraftment rates and acceptable TRM(23%) and rate of grade III-IV aGvHD, associated with durable remission in a proportion of patients with high-risk AL, specially in patients with AML transplanted in CR1.

PU046

A CASE OF A MYELODYSPLASTIC DISEASE DERIVED FROM A PREVIOUSLY HLA-HAPLOIDENTICAL TRANSPLANTED MARROW FOLLOWED BY A SECOND HLA-HAPLOIDENTICAL TRANSPLANTATION COMPLETELY MISMATCHED RESPECT TO THE FIRST

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Introduction: In the last years HLA-Haploidentical Bone Marrow Transplantation (Haplo-BMT) has been widely employed in high risk haematological maligniancies who have not an HLA matched donor. Here we describe a singular case of a patient who underwent Haplo-BMT for AML and developed a myelodysplastic syndrome 5 years after. He underwent a second Haplo-BMT from a different donor, whose HLA was completely different respect to the first donor. Clinical Characteristics: A 49 y male patient with high risk AML was submitted to Haplo-BMT from his brother in first complete remission in 2011. The outcome was favorable, no major complications occurred, only a mild self-limiting chronic GvHD was observed. After 5 years of complete remission, the patient presented worsening anemia and thrombocytopenia. A bone marrow aspirate was diagnostic for a three-lineage myelodysplastic syndrome, with 10% blast infiltration and a monosomal karyotype (45 XY; -7). The chimerism status evaluated on bone marrow and peripheral mononuclear cells remained "full donor" on multiple controls. We concluded for a second haematologic neoplasm

derived from donor marrow. To note, that the donor actually does not show haematological abnormalities. Treatment and results: Based on good clinical conditions, the patient underwent a second BMT from a different donor (his son) who was HLA-Haploidentical respect to him but completely mismatched respect to the first donor. Considering the chimerism status of the patient at the transplant, we were in the following situation: Haplo in GvHD direction, completely mismatched in rejection direction but also in Graft *versus* Leukemia (GvL) direction. In order to reduce the risk of graft failure we performed an ATG (7,5mg/Kg) priming 18 days before BMT. Anti HLA-antibody research revealed only a low title of anti-HLA of class-II. Conditioning regimen was a reduced-intensity TBF, GvHD prophylaxis was a conventional Cyclosporin-A, Mycophenolate and post-transplant Cyclophosphamide. No significant complication was observed during the procedure with a WBC and platelets engraftment at +29 days. Actually after 90 days he is in good clinical conditions, no infections, viral reactivations, GvHD nor other complications appeared. *Conclusions:* Here we report our singular experience of a double Haplo-BMT with two completely HLA different donors performed for a secundary neoplasm occurred in donor cells. In our knowledge there is no similar case reported in literature.

MAIN PROGRAM

BONE MARROW TRANSPLANTATION FOR APLASTIC ANEMIA: IMPROVING THE OUTCOME LONG TERM

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Bone marrow transplantation (BMT), is the treatment of choice for young patients with acquired severe aplastic anemia (SAA). The outcome has improved over time: in a EBMT analysis on 4511 patients, survival at 10 years was 54% in the period 1971-1998, and 68% in the period 1999-2009. Some issues are now given for granted, such as the conditioning regimen for patients receiving sibling transplants under the age of 30, which is cyclophosphamide 200, combined with ATG and marrow as a stem cell source. However many issues remain open, and among these, fludarabine for patients over the age of 30, peripheral blood grafts for patients receiving Campath, low dose radiation for older sibling grafts and for unrelated donor grafts, the use of cord blood as a stem cell source, and more recently the introduction of unmanipulated haploidentical transplants. An additional difficult issue is the age cut off, to select transplantation *versus* ATG for a given patient: contrary to what is believed, a recent study shows that older patients with SAA (>40 years of age) continue to have a high risk of mortality, also in 2015. Rejection remains a problem, typical of SAA patients, with high morbidity, and requires careful selection of the conditioning regimen. One of the primary issue for long term outcome is avoiding chronic graft *versus* host disease, and for this reason the use of either ATG or Campath is highly recommended for all patients. Patients age remains a strong predictor of outcome, and must be considered when designing therapeutic strategies. Early diagnosis and treatment, as well as long term monitoring, remain crucial steps for successful treatment of SAA.

HOW I TREAT AUTOIMMUNE HEMOLYTIC ANEMIAS

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Abstract: Autoimmune hemolytic anemia (AIHA) is a heterogeneous disease usually classified in warm, cold, and mixed forms, according to the isotype and thermal range of the autoantibody. First-line therapy for warm AIHA is based on corticosteroids (effective in 70-85% of patients, but curative is 20-30% only). Second-line therapy for refractory/relapsed cases includes splenectomy (responses in ~70% cases, which are however curative in ~20% only) and rituximab (effective in 70-80% of cases), which is becoming the preferred option. Thereafter, the choice is any of the immunosuppressive drugs (azathioprine, cyclophosphamide, cyclosporine), with a recent particular interest in mycophenolate mofetil. Additional therapies are intravenous immunoglobulins, erythropoietin, and danazol. For ultra-severe/refractory cases, last option treatments are plasma-exchange, high-dose cyclophosphamide and alemtuzumab. As regards cold agglutinin disease, rituximab is now recommended as first-line treatment. For refractory/relapsed cases combination therapy with bendamustine-rituximab or fludarabine-rituximab may be considered, and other promising options are upcoming (proteasome or complement inhibitors).

Autoimmune hemolytic anemia (AIHA) is a heterogeneous condition (estimated incidence 1-3 per 10^5/year, prevalence of 17:100,000) caused by autoantibodies directed against erythrocyte self-antigens, with or without complement activation. It's usually classified according to direct antiglobulin test (DAT) positivity and thermal characteristics of the autoantibody in warm (~70% of cases, DAT positive for IgG or IgG+C), cold (~20% of patients, DAT positive for C), mixed (7-8% of cases, DAT positive for IgG and C, with coexistence of warm autoantibodies and high titer cold agglutinins), and atypical cases (mainly DAT-negative and warm IgM) [1]. AIHAs can be primary or secondary to lymphoproliferative syndromes (10% of chronic lymphocytic leukemia (CLL) and 2–3% of non-Hodgkin's and Hodgkin's lymphoma), infections, immunodeficiency and tumors, and are described with increasing frequency following hematopoietic stem cell transplantation (HSCT) (about 4% of cases) or new drug administration, such as the program death 1 pathway inhibitor (anti-PD-1) nivolumab. Moreover, a clonal bone marrow lymphoproliferation was demonstrated in 75% and 90% of CAD by bone marrow biopsy or flow cytometry, respectively [2]. A precise diagnostic definition is fundamental, since therapy of warm and cold forms is quite different, and the identification of secondary forms is essential, since therapy should be firstly directed at the underlying disease. In particular, bone marrow biopsy and flow cytometry are recommended in warm and mixed cases relapsed after steroid therapy and in all CAD cases prior to therapy. Likewise, bone marrow biopsy is advisable in all multi-refractory cases to assess possibly underlying myelodysplasia, such as in the recently described idiopathic cytopenias/dysplasias of uncertain significance (ICUS/IDUS) [3]. The clinical severity of AIHA mainly depends on the autoantibody characteristics (class, thermal amplitude, and efficiency in activating complement), but also on the activity of the reticuloendothelial system (in spleen, liver and lymphoid organs), and on the efficacy of the erythroblast compensatory response. Reticulocytopenia, reported in 39% of children and ~20% of adults may be due to an anti-erythroblast reactivity and to the apoptosis of erythroid precursors, and may often represent a clinical emergency with an extremely high transfusion need. Moreover, all AIHA forms that show activation of the complement cascade (warm IgM, warm IgG+C, mixed, CAD with thermal range close to physiological temperatures) are clinically severe and potentially fatal. The mortality rate of AIHA is reported ~4%. Severe infections, mainly in splenectomized cases, acute renal failure, Evans syndrome and multitreatment (4 or more lines) are predictors of a fatal outcome [1]. The treatment of AIHA is still not evidence-based as there are no randomized studies and only a few prospective phase 2 trials [4-7].

Treatment of warm AIHA: Corticosteroids represent the first-line treatment and are usually given at the initial dose of prednisone 1-1.5 mg/kg/day for 1 to 3 weeks until hemoglobin is greater than 10 g/dL, and thereafter gradually and slowly tapered off during a period no shorter than 4-6 months. Side effects (reported as possibly less frequent when steroids are used as short-term bolus) should be treated rather than discontinuing the drug too early, since patients receiving low doses for more than 6 months have a lower incidence of relapse than those discontinuing the medication earlier. In patients with particularly rapid hemolysis and very severe anemia, or complex cases such as Evans syndrome, intravenous methylprednisolone at a 100-200 mg/day for 10-14 days or 250 to 1000mg/day for 1 to 3 days may be indicated. Corticosteroids provide a response in 70-85% of patients, however the estimated cure rate is about 20-30% only [1, 3, 4] (Table 1). Patients unresponsive to first-line therapy, early relapsed, or requiring inacceptable high doses (more than 10-15mg prednisone per day), need second-line therapy. Second-line treatment encompasses the choice between splenectomy and rituximab, nowadays the preferred choice, although

no prospective comparative trials are available. Splenectomy has an early response rate in ~70%, and a presumed curative rate in ~20% of cases, and the larger studies available report that it is performed in 10-15% of cases [1, 3, 4]. The drawbacks of this option are the lack of reliable predictors of outcome, the associated surgical complications, and above all overwhelming sepsis (3-5% of cases, with a mortality rate up to 50%, even after the recommended introduction of pre-operative vaccination against pneumococci, meningococci, and hemophilus). Revaccination against pneumococci and meningococci every 5 years and annual flu vaccine are now recommended, whereas the role and efficacy of antibiotic prophylaxis is controversial. Patient education, early administration of oral antibiotic therapy in case of fever, and prompt referral to hospital in the absence of response are fundamental to reduce the risk of overwhelming sepsis. Laparoscopic splenectomy is now preferred because of less trauma, fewer complications, shorter hospital stays, and an overall lower cost, but it should be performed by experienced operators. Regarding the timing of splenectomy, in the clinical practice it is generally performed not earlier than one year from the diagnosis; however, recent evidence indicates that splenectomy is associated with a poor prognosis when performed after multiple immunosuppressive therapies [3]. There is increasing awareness about the increased risk of thromboembolism and pulmonary hypertension after splenectomy, possibly due to procoagulant-derived microparticles, platelet activation, disturbance and activation of the endothelium, and persistent thrombocytosis. Thrombotic events occur in 10-15% of AIHAs, most frequently in severe cases with intravascular hemolysis and previous splenectomy, even in the absence of antiphospholipid antibodies, whose role is still a matter of debate [1]. In older and small series pulmonary embolism was fatal in all cases, whereas more recent studies show that pulmonary embolism or disseminated intravascular coagulopathy are not associated with a fatal outcome. Although prospective studies are required to give precise recommendations, an anti-thrombotic prophylaxis is advisable, particularly in severe cases associated with intravascular hemolysis and/or splenectomized. Additional factors to be considered in choosing splenectomy as preferred second-line treatment are younger age and wish to become pregnant. On the other hand, age older than 65-70 years, cardiopulmonary disorders, previous history or serious risk of thrombosis, hepatitis C, underlying immunodeficiency, lymphoproliferative, and systemic autoimmune conditions should be carefully considered before surgery [3] (Table 1). Rituximab is increasingly preferred among second-line treatments, being used in about 20-30% of steroid-refractory warm AIHA cases, with an overall response rate of about 70-80% (half of cases complete responses), comparable to that of splenectomy [1, 3, 4]. The median duration of response is 1-2 years, the disease free survival ~70% at one and ~55% at two years, and the median time to response is 4-6 weeks, although responses after 3-4 months are not uncommon. Moreover, responses are observed in monotherapy or in combination with corticosteroids/ immunosuppressants, after re-treatment in case of relapse, and regardless of prior therapy [8, 9]. Predictors of response seem to be younger age, shorter interval between diagnosis and treatment, and early administration as second-line therapy [1]. A recent prospective randomized trial showed that first-line treatment with glucocorticoids and rituximab resulted in ~70% of patients still in remission at 3 years compared with ~45% of those treated with steroids alone [6], strongly suggesting early combination therapy. Moreover, a prospective pilot study demonstrated that first-line treatment with low-dose rituximab (100 mg weekly×4) plus a short course of steroids compares favorably with conventional doses (overall response in 89% of cases, with complete response in 67%, relapse-free survival at 3 years in 68% of patients [5], again suggesting that this drug should be used early in the treatment of AIHA. Finally, rituximab was administered as early second-line treatment, no later than 7-10 days if steroids and transfusions failed to stabilize/ameliorate hemoglobin levels in very severe cases of AIHA, with a prompt resolution of the clinical emergency in most cases. The drug has a well-established safety profile (infectious events in roughly 7%), although rare cases of progressive multifocal encephalopathy, mostly in onco-hematologic conditions, hepatitis B reactivation and other viral infections have been reported. To prevent hepatitis B reactivation both after rituximab and prolonged steroid therapy antiviral prophylaxis is now recommended [8, 9] (Table 1). As regards conventional immunosuppressive drugs (such as azathioprine, cyclophosphamide, cyclosporine), response rates are reported in about 40% of cases, but partially attributable to concomitant steroid administration [1, 3]. Although their use may be associated with serious side effects immunoppressants are still used in the clinical practice (~25% of cases), and may be considered mostly as steroid-sparing agents when splenectomy is not feasible and/or rituximab unavailable. Mycofenolate has raised increasing interest because of proven effectiveness in ~90% of refractory immune cytopenias in children, and in post-hematopoietic stem cell transplant AIHA together with rituximab, although larger confirmatory studies are needed to give precise recommendations [3] (Table 1). Concerning other treatment options, it is worth reminding intravenous immunoglobulins (IVIG), which are frequently used in children for their low incidence of adverse effects and responses in about 40-50% of cases. Moreover, danazol has been reported effective in ~40% of cases in older studies, not confirmed in more recent series. Finally, erytropoietin has been successfully used in patients with therapy-refractory AIHA, and may be indicated particularly in the presence of reticulocytopenia. Moreover, it was proven useful in patients with very severe presentation, being able to reduce transfusion need and avoid hemolysis related to overtransfusion. For the few patients refractory to previous therapies, it is worth mentioning high dose cyclophosphamide (50 mg/kg/day for 4 days) followed by granulocyte colony-stimulating factor, which was reported effective in 5/8 patients with highly refractory warm AIHA. Alemtuzumab induced good responses, particularly in CLL-associated AIHA, but is associated to high toxicity. More recently, bendamustine and rituximab combination has been reported highly effective in CLL-associated AIHA (81% for AIHA and 77% for CLL), with a median time to next treatment of 28 and 26 months for AIHA and CLL, respectively. Ofatumumab has been successfully used in a case of CLL-associated warm AIHA refractory to rituximab. Information on HSCT is limited, this option being used in very severe and ultra-refractory cases (mostly Evans syndrome). The overall complete remission rate is approximately 60% in allogeneic and 50% in autologous HSCT, and a continuous remission is reported in 3/7 allogeneic and 1/7 autologous HSCT, with a transplant-related mortality of approximately 15%.

Table 1. Treatment options for warm AIHA.

Treatment	Approximate response rate	Duration of sustained response	Toxicities
First-line			
Corticosteroids (i.e. prednisone 1 mg/kg/day p.o.)	~75-80%	20-30% once the drug is discontinued, a further 50% require maintenance doses, and approximately 20-30% needs additional second-line therapies	hypertension, diabetes, weight gain, anxiety, osteoporosis, gastro-intestinal ulcers, immune-suppression, psychosis, cataracts
Second-line			
Splenectomy	~70%	20-60% after a 4-7 years follow-up	Surgical, infective, and thrombotic complications, particularly in patients over 65 years
Rituximab			
standard dose (375 mg/m^2/wk x 4)	~70-80%	>3 years in some cases	Mild to moderate infusion-related side effects; grade 4 neutropenia (roughly 2%) and infectious events (roughly 7%).
low dose (100 mg/wk x 4 wk)	~80-90%	~68% at 2 and 3ys	
Third-line			
Immunosuppressive drugs (azathioprine, cyclophosphamide, cyclosporine)	~40-60%	Not available	Side effects vary with the drug (i.e. severe neutropenia with infection, renal or liver impairment)
Mycophenolate mofetil	65-90% (50% complete)	Not available	Immunosuppression without serious side effect
Last-option			
high-dose cyclophosphamide 50 mg/kg/day for 4 days	60% (9 pts studied)	Not available	Not reported
Alemtuzumab 10-30 mg IV or SC three times week for 8-12 weeks	100% (12 pts studied)	100% after 1 year follow-up	Not reported in this studied. Various rates of immune suppression and infections depending on dosing, number of cycle, and underline disease
Other options			
Ivlg 0.4 g/Kg/day for 5 days	40-50% (data from a single report, 1993)	Generally transient	Very rare serious side effects (e.g. hypersensitivity, infectious contaminations, thrombotic events)
Erythropoietin 100-200 U/Kg weekly	90-100% (mostly associated with other treatments)	Not available	Arterial hypertension, thrombo-embolism
Danazol 100-200 mg/BID	60-70% (data from a single report, 1990)	5 years	Androgen like effects, hepatic dysfunction

Treatment of CAD: The decision to treat CAD should be reserved to patients with symptomatic anemia, transfusion dependence, and/or disabling circulatory symptoms. In fact, non-severe asymptomatic forms may require only protection against cold exposure, and occasional transfusion support during winter [2]. Steroids are effective in a

small fraction of cases (14-35%) and usually at unacceptably high doses. Therefore, this treatment, although still used in the clinical practice, is now discouraged. Concerning conventional cytotoxic immunosuppressive drugs, monotherapy with chlorambucil or cyclophosphamide has shown some beneficial effect in small series (16% of cases); at variance, no convincing responses were observed in the few patients treated with azathioprine, interferon- or low-dose cladribine. Splenectomy is usually ineffective, due to the fact that clearance of C3b-opsonized erythrocytes primarily occurs in the liver. Rituximab is now recommended as the first-line treatment of CAD [2], although complete and sustained remissions are uncommon. The drug is effective in ~60% of cases (5-10% CR), with a median time to response of 1-2 months, and a response duration of 1-2 years [1, 2]. Responses are observed following a second and even a third course in relapsed cases (Table 2).

Table 2. Treatment options for cold agglutinin disease (CAD).

Treatment	Approximate response rate	Duration of sustained response	Toxicities
First-line			
Rituximab (standard dose (375 mg/m²/wk x 4)	~60% (5-10% complete)	1-2 years	Mild to moderate infusion-related side effects; grade 4 neutropenia (roughly 2%) and infectious events (roughly 7%).
Corticosteroids (only for very short period during the diagnostic phase)	14-35% At unacceptable high doses only	Not available	Hypertension, diabetes, weight gain, anxiety, osteoporosis, gastro-intestinal ulcers, immune-suppression, psychosis, cataracts
Second-line			
Rituximab (375 mg/m² day 1) + **Bendamustine** (90 mg/m² day 1 and 2) for 4 cycles at 28 days interval	71% (40% complete)	10-percentile of response duration not reached after 32 months	Grade 3-4 neutropenia in 33% of cases, infections in 11%, dose reduction required in 29% of cases
Rituximab (375 mg/m² day 1) + **Fludarabine orally** (40 mg/m2 on days 1–5) for 4 cycles at 28 days interval	76% (21% complete)	Median 6.5 years	Grade 3-4 hematologic toxicity in 41% of cases, infection grade 1-3 in 59%, dose reduction required in 45% of cases.

In attempt to improve response rates, combined treatment with rituximab and fludarabine orally (40mg/m^2 on days 1–5) was proven to induce higher response rates (76% of cases) and sustained remissions (estimated median response duration 6.5 years) [10]. Since hematological toxicities and infective complications were common, this regimen is suggested for cases refractory to 1-2 courses of rituximab. Based on the assumption that CAD is associated with a bone marrow clonal B-cell lymphoproliferation, a recent prospective trial was conducted with rituximab (375 mg/m^2 day 1) and bendamustine (90 mg/m^2 day 1 and 2) for 4 cycles at 28 days interval. This treatment was proven effective in 71% of cases (40% complete and 31% partial responses), with an interesting response duration and an acceptable safety profile, suggesting that it may be considered in first line for patients with CAD requiring therapy [7] (Table 2). As regards new experimental approaches, improvement of anemia has been observed in 2 patients following monotherapy with bortezomib, and in 2 cases after administration of eculizumab, the monoclonal anti-C5 antibody licensed for paroxysmal nocturnal hemoglobinuria [2, 3]. Further evidence for the potential use of complement inhibitors is the findings that TNT003, a mouse monoclonal antibody targeting complement protein C1s, prevents the induction of *in vitro* hemolysis by cold agglutinins. Further complement inhibitors, such as compstatin Cp40, a low-molecular-peptide that prevents cleavage of C3, and TT30, which prevents C3 convertase activation, have shown promising effects on complement-mediated lysis of erythrocytes in PNH, and may be potential treatments suitable for acute complement-mediated hemolysis. Finally, it is worth mentioning paroxysmal cold hemoglobinuria (PCH), which is characterized by acute intravascular hemolysis mediated by the Donath–Landsteiner biphasic hemolysin. This IgG antibody binds to erythrocytes at low temperatures and causes complement-mediated hemolysis at 37°C, and is directed against the P blood group system. In the past PCH was mainly associated with syphilis, and now usually follows viral and bacterial infections, including Mycoplasma pneumonia. PCH is usually a self-resolving disease, although deaths have been reported; the few severe cases may require transfusions, PEX, and steroid treatment, whose effectiveness is difficult to evaluate because of the transient nature and the rarity of the disease. As regards mixed forms (~7-8% of all AIHAs), there are no clear indication whether to treat them as warm or cold forms, although their more severe onset and refractoriness to several therapy lines, suggest aggressive and combined early therapy with steroids and rituximab [1].

Supportive therapy: Patients with AIHA may require red blood cell (RBC) transfusion to achieve and/or maintain clinically acceptable hemoglobin values until specific treatments become effective. The decision to transfuse should depend rather on the patient's clinical status and comorbidities (particularly ischemic heart or severe pulmonary disease) than on the hemoglobin level. However, blood transfusion should never be denied to patients, particularly in cases with acute onset, rapid worsening of anemia, and hemodynamic instability. Generally, values lower than 6 g/dL deserve support [1, 3, 4] even though no truly compatible units can be found. ABO- and RhD-matched red cell concentrates can be anyway safely administered in urgent cases if alloantibodies are reasonably excluded based on the previous transfusion and/or pregnancy history. In less urgent cases an extended phenotyping is advisable and the best compatible red cell units should be selected. It is worth reminding that alloantibodies occur in 12%-40% of AIHA patients, and may be responsible for severe hemolytic reactions. Undetected alloantibodies may cause increased hemolysis following transfusion, which might be falsely attributed to a worsening of AIHA. In addition, leuko-depleted red cells are nowadays recommended in AIHA patients, to minimize risks of febrile non-hemolytic reactions due to anti-leukocyte antibodies. Erythrocyte transfusions can safely be given in CAD, provided appropriate precautions, such as keeping the patient and the extremity chosen for infusion warm, and using of an in-line blood warmer. Finally, RBCs should also be administered slowly and possibly not exceeding 1 ml/kg/h. Overtransfusion (with an increased mass of RBCs available for destruction) should be avoided, particularly in elderly patients. As regards plasma-exchange (PEX) the results reported in the literature are controversial, with favorable effects generally short-lived, so that it was defined a "heroic or last–ditch efforts on behalf of a patient". However, PEX may be useful in critical and very severe AIHA, both in stabilizing hemoglobin and in reducing hemolysis, particularly in the presence of inadequate reticulocytosis. In patients with CAD, plasmapheresis may be useful in acute hemolytic crisis and before surgery requiring hypothermia, although its effect is transient.

Conclusions: The therapeutic battery now available for steroid-refractory warm primary AIHA is certainly broader than in the past, although the best sequence of second-line therapy is not clearly established. Few years ago the expert opinions recommended splenectomy, rituximab, and thereafter any of the immunosuppressive drugs [4]. More recently, rituximab is suggested at an earlier point, particularly in severe cases and in children aged <5-6 years, or when splenectomy is refused or contraindicated for comorbidity or age [3]. As regards CAD, rituximab is now recommended as first-line, and steroids are justified only in a very early and short phase, just during diagnostic procedures. Refractory CAD cases have still few therapeutic options, although new drugs are upcoming [2]. In general, the choice of second-line therapies is still mostly determined by the physician personal experience, the patients' preference, and the availability of the new drugs, rather than on controlled trials that are, of course, advisable. Moreover, these is need of target therapies that specifically mark the variety of immunologic mechanisms of the disease in the different AIHA patients (autoantibodies, cytotoxicity, phagocytosis, complement, and bone marrow insufficiency), to promote a curative and less toxic approach to autoimmunity.

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QUALITATIVE AND QUANTITATIVE FEATURES OF MONOCYTIC SERIES IN PREDICTING REACTIVE VERSUS CLONAL MONOCYTOSIS

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Monocytes are members of the mononuclear phagocyte system, which also includes two other major subtypes, the dendritic cells (DCs) and the macrophages (Guilliams, 2014). Cells of the monocyte/macrophage lineage are derived from hematpoietic stem cells oriented towards granulocyte/monocyte differentiation. The migration of monocytes into tissues leads to their transformation into macrophages, a reversible process mediated by numerous biologically active substances, mainly as a response to local factors and conditions. Monocytes have long been considered to be the main source of tissue macrophages: however, recent data demonstrated tissue-resident macrophages that develop during embryogenesis. Monocytes represent 1% to 9% of peripheral leukocytes and, like neutrophils, are clustered in a marginated and a circulating pool, with an average ratio of 3.6:1. This pattern accounts for the strong increase of monocytes count within minutes by stress or exercise followed by a rapid return to baseline levels. Trafficking into and through blood is finely tuned in response to recruitment signals. Monocytes are not a homogeneous population. Based on the flow cytometric expression of CD14/CD16, they can be distinguished into classical MO1 (CD14+/CD16–), intermediate MO2 (CD14+/ CD16+) and non-classical MO3 (CD14–/CD16+) fractions, with MO1 constituting the most represented subset (85%) in healthy conditions (Tallone, 2011). Classical monocytes are the principal source of DCs, while all subsets can differentiate to macrophages.

Morphology: Mature monocytes are the largest cells normally present in the peripheral blood (diameter 15-20 m). The nucleus occupies about half of the cell; it is often slightly eccentric, variable in shape (round, oval, indented, renifom, lobulated, folded, bean- or fetus-shaped); the chromatin is spongy, reticular, pale, composed of a network of filaments which interweave in a very characteristic loose network: this chromatin structure represents one of the most useful morphological criteria to differentiate them from large lymphocytes. The cytoplasm is voluminous, from blue to greyish with a pink tinge that is more or less prominent depending on the quantity of numerous, minute, irregular azurophilic granules: these hardly ever individually reach the threshold of visibility, but they give to the cytoplasm a sandy, ground-glass, dusty appearance. Vacuoles are often present and may be numerous, particularly after a prolonged period of storage in the EDTA tube. Mature monocytes which can be seen in a bone marrow aspirate are generally similar to those in the circulation, but they appear smaller: the nucleus has apparently more compact chromatin and the cytoplasm is less voluminous and more basophilic with fewer vacuoles. By definition the absolute monocyte count is above 1×10^9/l. In adults chronic infections, autoimmune disorders and chronic myelomonocytic leukemia are the most frequent causes of monocytosis.

Monoblasts: are usually large, with cytoplasm that is usually abundant and weakly basophilic, a nucleus that is regular and round or oval, dispersed, fine and delicate chromatin and often evident nucleoli. Between these two extremes one must site the other, less typical cells of the monocyte lineage, evaluating precisely their shape and structure. In particular, it is the identification of delicate and finely dispersed chromatin, regardless of the shape of the nucleus, the colour of the cytoplasm that is more basophilic than in mature monocytes, and the possible presence of granules, that identifies a promonocyte, which in this context must be included in the blast count, together with granular and agranular myeloblasts and monoblasts. *Promonocytes* can be described as cells that have the chromatin structure and nucleolus of a monoblast, while the nuclear shape may be irregular and the cytoplasm may have staining characteristics more similar to those of monocyte, and can contain granules that are more evident than those of mature monocytes. (Goasguen, 2009). Those cells belonging to the monocytic series that do not fit the morphological features of mature monocytes, promonocytes and monoblasts should be included into the group of *Immature/Atypical/Abnormal monocytes* (Zini, 2010) (Figure 1). Peripheral smear review is mandatory in cases with monocytosis. Morphology is required to assess maturity in monocytes and to classify them in a proper way as mature monocytes, immature/atypical monocytes, promonocytes and monoblasts. This assessment is crucial since promonocytes have to be considered as blast equivalents in the right context and should be included in the blast count. Additionally, assessment for dysplasia is required, as its presence can support the final diagnosis of hematologic malignancy.

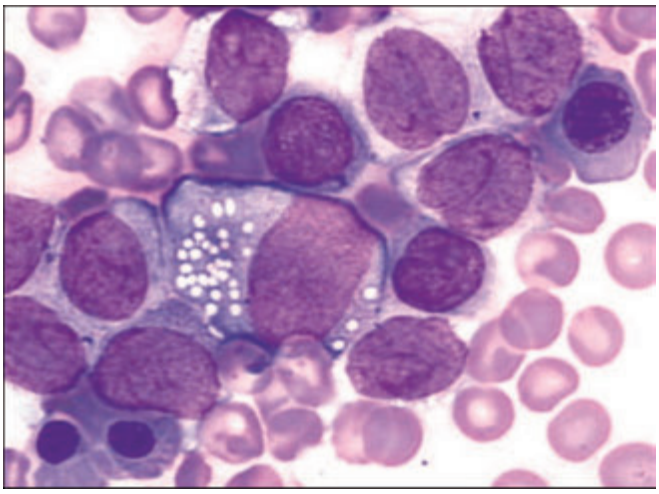

Figure 1. (MGG,100x). Chronic myelomonocytic leukemia with massive bone marrow invasion by much less mature cells, bone marrow aspirate. The largest cell, vacuolated with fine chromatin and a regular nuclear outline, can be classified as a monoblast, as can the smaller cell adjacent to it on the left. The three cell high up on their right, however, are promonocytes by virtue of their folded nuclei. Immediately beneath, an atypical monocyte with scanty cytoplasm. Up right one polychromatophilic erythroblast and bottom left, two orthochromatic erythroblasts.

Cytochemistry: Esterase are a group of hydrolases localized in cells of different lineage: nine isoenzymes have been described in leukocytes grouped into two groups. The "specific" esterase of granulocytes can be demonstrated by means of naphtol AS -D (or ASD), chloroacetate (NASDA and NADA) while the so called "non specific" are sensitive to sodium floride (NaF), are found in monocytes, megakaryocytes and platelets and are demonstrated by alpha-naphtol esters (acetate and butyrate) (ANAE). Combined butyrate esterase and chloroacetate esterase technique permits in the same smear to identify both granulocytic and monocytic components (Dacie, Lewis,1991) (Figure 2).

Reactive monocytosis: Infection is the most common cause of reactive monocytosis (Figure 3). Several infectious diseases, such as of tuberculosis, brucellosis, typhoid fever, leishmaniosis, rickettsia diseases, subacute bacterial endocarditis, syphilis, tonsillitis, recurrent liver abscesses, candidiasis, viruses, in particular cytomegalovirus and varicella zoster virus, are described to be associated, although infrequently, with monocytosis. Other common causes include inflammatory conditions, such as collagen vascular disease (rheumatoid arthritis, temporal arteritis, systemic lupus erythematosus, myositis, periarteritis nodosa), chronic neutropenia, splenectomy, hemolytic anemia, immune thrombocytopenic purpura. An unexplained expansion of monocytes should raise the possibility of occult cancer. Hematological malignancies can also be associated with monocytosis, sometimes with prognostic impact: this is the case of diffuse large B cell lymphoma and Hodgkin lymphoma. Other disorders associated with monocyte pro-

liferations include alcoholic liver disease, tetrachloroethane poisoning, and Langerhans cell histiocytosis. Monocytosis is also a frequent finding at the time of delivery. Psychiatric depression can be associated with a increase in neutrophils and monocytes. Finally, monocytosis may occur as a compensatory event in association with congenital or drug-induced neutropenia (Lichtman, 2016). Once monocytosis is confirmed, the next diagnostic step should be the assessment of the clonal or reactive nature of the proliferation. A detailed history of symptoms can provide clues to a diagnosis. If clinical correlation fails to explain a case of a sustained peripheral blood monocytosis, a bone marrow specimen (aspirate and biopsy) with cytogenetic karyotyping is required. It has been shown that multiparametric flow cytometry, by identifying different distributions of monocyte subpopulations, can be very useful in distinguishing clonal proliferations from reactive expansions.

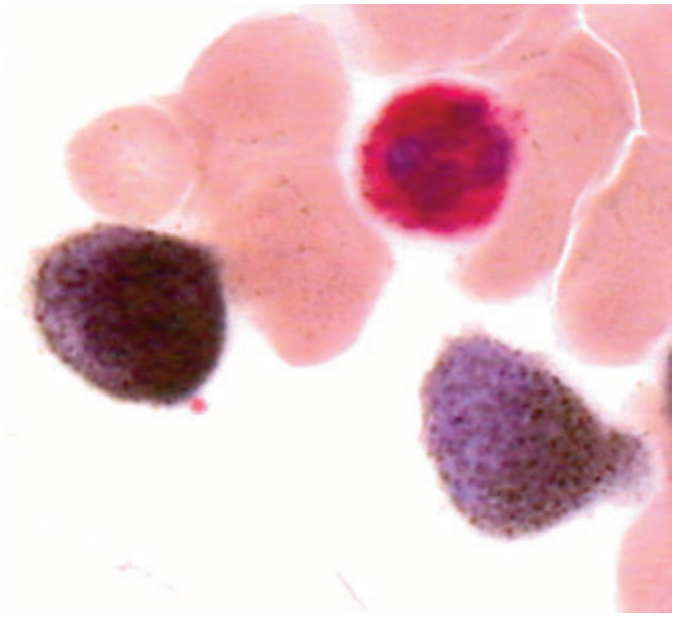

Figure 2. (Double esterase stain, 50x). Cytochemistry of cells of monocyte lineage in chronic myelomonocytic leukemia, peripheral blood: the monocytes and their precursors show the typical diffuse black granular cytoplasmic positivity for alpha naphthyl acetate esterase; the neutrophil, on the other hand, shows the red staining indicating positivity for chloroacetate esterase.

Neoplastic monocytosis: Several primary hematologic neoplasms harbor neoplastic monocytes including Chronic myelomonocytic leukemia (CMML), acute myeloid leukemia with monocytic differentiation, and myeloproliferative neoplasms such as chronic myelogenous leukemia (CML). While the peripheral smear of CML is usually characteristic, the findings in CMML and in acute leukemia with monocytic differentiation may be similar in peripheral blood. The bone marrow is often the site of increased immature monocytes. For this reason unexplained sustained peripheral blood monocytosis, or presence of monocyte precursors, deserve a bone marrow specimen (aspirate and biopsy) with cytogenetic karyotyping. In selected cases, high-throughput sequencing technologies, through the recognition of molecular signatures typical of neoplastic monocytes, can help to overcome the diagnostic impasse in demonstrating clonality. Morphology is critical to enumerating monoblasts and the blast-equivalent promonocytes, and to distinguish them from mature monocytes, both reactive and atypical. Cytochemical staining may be useful since it improves the sensitivity over morphology alone for the detection of monocytic cells. Based on World Health Organization (WHO) 2008 criteria, the diagnosis of CMML requires at least three months of unexplained monocytosis (>1.0×10^9/l) with dysplasia in at least one myeloid lineage. According to the WHO 2016 revised classification (Arber, 2016) diagnosis of CMML requires the detection in PB of an absolute monocyte count above 1×10^9/l and/or a percentage of monocytes ≥10% in the differential to identify the group of oligomorphic CMML (Geyer, 2017). By definition the blast count (including myeloblasts, monoblasts and promonocytes) should be <20%. Three subgroups identified by the blast amount are proposed for a better prognostication: CMML-0 (PB blasts<2%, BM blasts <5%), CMML-1(PB blasts 2% to 4%, BM blasts 5% to 9%), CMML-2 (PB blasts 5% to 19%, BM blasts 10% to 19% and/or when any Auer rods are present). Flow cytometry is capable of unveiling subtle immunophenotypic aberrancies, but it is not able to reliably distinguish promonocytes (blast equivalents) or monoblasts from mature and/or immature/atypical/abnormal monocytes. Because of this, flow cytometry is the least reliable modality to assess for a neoplastic monocytic disease. Reactive monocytes may have aberrant expression by flow cytometry, particularly CD56 and/or diminished human leukocyte antigen–DR, CD33, and CD13. The finding of two or more aberrancies favors a neoplastic process, though this is not specific. It is reported that a high fraction of CD14+/CD16– monocytes distinguishes chronic myelomonocytic leukemia (CMML) from reactive causes, with a 67% of sensitivity and a 100% of specificity for CMML (Xu, 2005). For this reason the cytomorphological diagnosis of MDS/MPN and MPN must be based, inescapably, more than in other conditions, on an accurate morphological assessment of the peripheral blood. The erythroid series, on the other hand, is in general better preserved in CMML than in MPN, with the percentage of erythroid cells generally being above 10% and the myeloid:erythroid ratio being less elevated than in CML. Moreover dysplastic features are usually absent in CML cases. In the setting of CMML, when dysplasia is absent, the presence of an acquired cytogenetic or molecular clonal abnormality is required. Other disease-defining translocations, notably *BCR-ABL1* fusion must not be present; notably, the presence of e1a2 transcript is associated with monocytosis and a poor prognosis. Cases presenting with eosinophilia should be excluded from the diagnosis of CMML in presence of *PDGFRA, PDGFRB* or *FGFR1* raerrangements or of *PCM1-JAK2* fusion gene. Moreover a well-documented prior diagnosis of a Myeloproliferative neoplasm as well as of other types of MDS/MPN, excludes the diagnosis of CMML. Finally, according to the presence/absence of mutations in the *RAS/MPK* pathways (Cervera, 2014), two subtypes of CMML are acknowledged with a different prognostic significance, based on the white blood cell count (WBC) at diagnosis: the "dysplastic type" and the proliferative type" presenting at diagnosis with a WBC below 13×10^9/l, and ≥13×10^9/l, respectively. The proliferative subtype has a worse prognosis impacted by presence of *RUNX1* mutations.

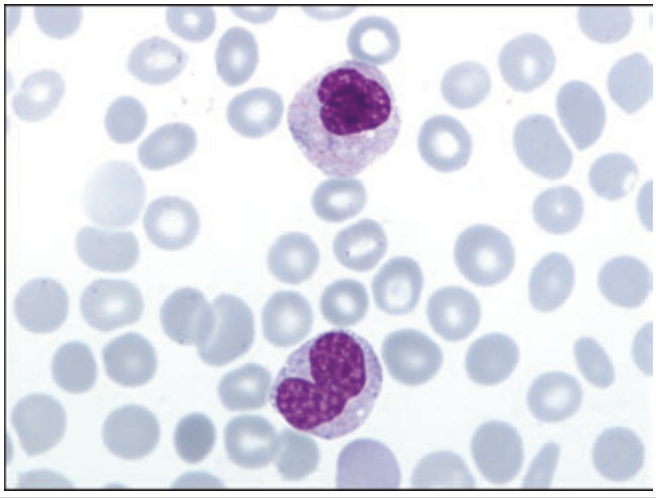

Figure 3. (MGG, 100x). Monocytosis in a patients with systemic lupus erythematosus, peripheral blood. The two monocytes are morphologically mature.

Conclusions: Monocytosis is a common and sometimes diagnostic finding. Accurate diagnosistic work-up equires the integration of clinical history, morphology, and ancillary tools, such as flow cytometry, citogenetics and molecular tecniques for the differential diagnosis between the many entities which can be associated with monocytosis.

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INDICATIONS TO ALLOTRANSPLANT IN ACUTE MYELOID LEUKEMIA

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Acute Myeloid Leukemia (AML) is the first indication to allogeneic hematopoietic stem cell transplantation (HSCT) representing roughly one third of whole activity. Decision to proceed to allogeneic HSCT in AML in first complete remission (CR1) is largely based on cytogenetics and molecular characteristics, as recently stated in the recommendations from the ELN panel (1). However two additional considerations should be made when deciding about allotransplant: the first is that the molecular heterogeneity of AML is larger than explained by those genes currently used to establish the therapy and we still don't know exactly if and how the genomic landscape of AML can increase the prognostication of AML; secondly, there are additional factors to AML genetics which should be considered such as the donor availability and type, the presence of patient comorbidities, the intensity of conditioning regimen and the presence of minimal residual disease (MRD). Allotransplant in CR1 is currently indicated for high risk patients and not indicated in all favourable risks (1). A meta-analysis comparing allogeneic *vs* non allogeneic approach showed that the benefit was restricted to non favourable risk patients. For the intermediate risk patients the careful evaluation of the ratio between pros and contras plays a crucial role and all the above mentioned factors become extremely important for this purpose. However, nowadays, indication to allogeneic HSCT in CR1 in the intermediate risk is still a matter of debate and autologous transplant gains space especially in MRD negative patients. Donor availability is not yet a limiting factor as it was in the past, because the increasing use of mismatched related and unrelated transplants and, in a smaller magnitude, of cord blood transplants are filling an import gap between the need and the availability of a suitable donor. That has led to dramatic changes on the hierarchy of donor selection, although a prospective controlled comparison is still lacking and deeply awaited. However the expansion of donor availability definitively abrogated the design of *donor vs no donor* studies toward a *transplant vs no transplant* approach, even in prospective trials (Venditti A. et al, oral presentation at EHA 2017). Haplotransplants grew in a tumultuous way in the last years, especially in Italy, giving the possibility of finding a suitable donor to almost all fit patients. Currently, HLA sibling donor and 10/10 unrelated donor remain the standard options for allotransplant in AML (2). There are no enough evidences about HSC source advantage between bone marrow (BM) and peripheral blood (PB) stem cells apart from preferring PBSC in case of advanced disease or reduced intensity conditioning (RIC) to counterbalance the higher relapse risk. Cord Blood (CB) transplants, which are rapidly decreasing in the worldwide activity, although with different magnitude relative to individual Countries, showed an important anti leukemic effect in MRD positive patients. No definitive statements on the hierarchy of donor choice can be done according to graft *vs* leukemia effect because of lack of prospective trials. The median onset of AML occurs in the seventh decade of human life and it implies that allogeneic HSCT cannot be offered to all the AML patients. The ELN classification groups patients as those being fit to undergo intensive chemotherapy and those not. Similarly, in the field of allogeneic HSCT, the fitness to transplant can be evaluated by the analysis of comorbidities, incorporated into the comorbidity Index (HCI); the index (3) is very powerful to dissect different risk stratification of patients, it has been largely validated and can be also of help to tailor the intensity of conditioning. The HCI has been developed initially for older patients and then validated across all age limits, suggesting that older patient with low HCI can tolerate higher dose of preparative regimen while a younger patient with HCI ≥3 should have the doses of chemotherapy reduced. In agreement with this, American guidelines stated that age itself cannot be an absolute contraindication to allogeneic HSCT and concordantly the number of transplants in patients above 60 years is increasing in the last years, according to the ageing of the overall population, at least in the most industrialized countries. In addition to HCI, geriatric assessment has been shown to be an independent factor predicting non-relapse mortality (NRM) in elderly patients. Another important score to be considered for predicting NRM in acute leukemias is the new ALEBMT-ADT score (4) deriving from a data mining approach (machine learning algorithm), which returns a continuous probabilistic score for the prediction of transplant mortality, freely available online (http://bioinfo.lnx. biu.ac.il/_bondi/web1.html). The issue of age and comorbidity is strictly connected to the intensity of conditioning (3). The use of RICs extends the applicability of a potentially curative treatment to a wider proportion of AML patients according to age and comorbidities; the lower NRM, anyway, is essentially counterbalanced by a higher relapse risk and the overall effect in different setting is still a highly controversial issue. Several analyses from retrospective studies, metaanalysis and prospective studies comparing RIC and myeloablative conditioning regimen (MAC) in AML and myelodisplastic (MDS) patients found similar overall survival, being the former associated with increased relapse rate and reduced NRM. Focusing the attention to randomised studies, one (5) didn't find significant differences between RIC and MAC but the trial was prematurely closed because of low accrual. Scott et al published recently (6) the results from the cIBMTR study CNT0901, where patients with AML and MDS with less than 5% blasts were randomly assigned to RIC or MAC. The primary endpoint of the study was the 18month-OS and the sample size was calculated accounting an overall survival gain for RIC arm of 15% (from 45% to 60% at 18 months). The study was prematurely closed because an advantage for EFS in the MAC arm; that interim analysis on EFS was not pre planned in the study. Survival at 18 months was 77.5% in MAC *vs* 67.7% in RIC (p=0.71) resulting in a significant difference only in AML patients (76.4% *vs* 63% p=0.035). Relapse was 13.5% *vs* 48.3% and NRM was 15.8% *vs* 4.4%, respectively. Relapse-free survival at 18 months resulted 67.8% *vs* 47.3% (p<0.01). The reason for the premature closure was the expected benefit for MAC due to a difference, higher than estimated, in relapse risk which translated into a poorer OS only in AML patients. The conclusion of the study is that for patients who can tolerate a MAC regimen, the full intensity of preparing regimen should be maintained. In particular, patients with AML with high disease risk and low HCI are the best candidates to experience the greater benefit of a MAC. The most used myeloablative conditioning regimens are Busulfan-Cyclophosphamide (Bu-Cy), Busulfan-fludarabine (Bu-Flu) and total body irradiation-Cyclophosphamide (TBI-Cy). The first one seems, in a retrospective analysis, to be at least equivalent to EDX-TBI. An Italian prospective randomised study compared in middle aged AML patients in CR the standard Bu-Cy *vs* Bu-Flu suggesting that the latter should be the new standard for this setting (7). MRD is an important help leading the choice to proceed or not to allogeneic HSCT, especially in the intermediate risk. The MRD level pre allogeneic HSCT correlates with survival and relapse and recently it has been confirmed also by a metanalysis. A seminal paper in this field has been recently published by Ivey et al (8) in patients being NPM-1 mutated and undergone intensive chemotherapy. The study evaluated the role of MRD, measured as NPM-1 transcript levels after two chemotherapy cycles, as a predictor of relapse. The results of the study showed that MRD is predictive of relapse (82% *vs* 30% at three years) and it repre-

sents also an independent prognostic factor, (independent from those genetic markers used for the indication to allogeneic HSCT); this finding translated into lower survival probability in MRD positive patients (24% *vs* 75% at three years). In addition the study found that among patients with high risk genotype (DNM3A, FLT3-ITD) MRD segregates a substancial group (79%) with good prognosis (76% OS). The study didn't find a significant benefit of allogeneic HSCT in MRD positive patients. It should be underlined that in this study only one third of patients with MRD positivity underwent allo HSCT within three months and it can be an important limitation which need further confirmation. In the study from Balsat *et al.* (9) post induction NPM-1 MRD monitoring was evaluated to test its role as predictive factor for allogeneic HSCT benefit: patients were allocated to allogeneic HSCT in CR1 if not belonging to the favorable ELN risk group. The trial confirmed that the value of NPM-1 MRD is an efficient early monitoring marker; it is also a prognostic factor regardless of FLT3 status (in bivariate analysis with FLT3-ITD and NPM1-MRD in PB only a >4 log reduction was significantly protective of relapse) and finally that it is a good predictor of allogeneic HSCT benefit which could be demonstrated, in this study, only for poor responder (MRD positive) patients. A well standardized assessment will be helpful to understand further the true subset of patients which can benefit from an allogeneic HSCT. In multivariate analysis a <4-log reduction of, the presence of FLT3- ITD, and an abnormal kariotype were independent prognostic fctors for relapse and survival. The GIMEMA trial 1310 has been designed adapting the intensity of post remission treatment to the genetic risk stratification, allocating into allogeneic HSCT all high risk patients, no patients belonging to favourable risk and only MRD positive patients for the intermediate risk while MRD negative patients were allocated to autologous HSCT. Only preliminary data has been recently presented and definitive analysis is strongly awaited. Focusing on transplant in FLT3-ITD AML patients, allotransplant in CR1 is highly indicated especially when the allelic ratio is high or DNMT3A mutation is also present. In these patients the prognosis is poor and can be partially improved by allogeneic HSCT which is characterized by a higher relapse risk, after both MAC and RIC transplant.

Transplant in CR2 and refractory/active disease: Relapsed patients should be considered for allogeneic HSCT. Evaluation of genetic profile should guide the choice of salvage therapy to achieve a CR2. Patient achieving a CR2 can experience survival probability of 40-50%. Given that there are no satisfying salvage options for refractory/relapsed AML, allogeneic HSCT remains an option to be given. Several points are still to be clarified: definition of primary refractoriness, timing of refractoriness, role of genetics, intensity and type of conditioning. Two retrospective analyses from EBMT and GITMO confirmed that age, disease burden at transplant, <2 cycles of chemotherapy and adverse genetic profile are adverse factors for outcome and that the majority of patients ultimately died because of relapse. Survival is poor and no significant differences are found between busulfan-based and TBI-based conditioning regimens. An interesting approach is the use of intermediate regimens closely before a FLAMSA cycle of chemotherapy. Schlenk *et al.* (10) recently published the result of five 1st line trials where 3324 patients were enrolled and 1307 relapsed after chemotherapy (953), autologous HSCT (79) and allogeneic HSCT (275). After relapse the salvage treatments were: intensive chemotherapy (907), direct allogeneic HSCT (100), non intensive chemotherapy (62), DLI (40), investigational therapies (21). 187 patients received palliative care with a 24 month-survival of 3.7%. Patient receiving a salvage therapy showed instead a 24 month-survival of 27.3%. The probability to achieve a CR2 with standard chemotherapy was very low. Overall, 40% underwent allogeneic HSCT 100 directly, 437 after intensive chemotherapy. Response to salvage therapy is correlated with genetic markers (CBF-AML, biallelic CEBPA, duration of CR1, absence of negative genetic markers and FLT3-ITD). Allogeneic HSCT had the highestchance to cure.

Conclusions: The genetic profile of AML is increasingly being studied to explain the heterogeneity of this disease and the need of tailored approaches. The approach to AML therapy should take into account since the beginning of the patient's story, the genetic features as well as the MRD monitoring and the transplant characteristics in terms of donor type, comorbidities and conditioning: the use of comorbidity index ameliorates the risk estimate but additional scores, such as the ALEBMT-ADT score, should be extensively encouraged, as well as an integrated approach, as suggested by Cornelissen et al (2) for a better evaluation of the balance between transplant and disease risk. The majority of the studies on AML are not powered enough to answer definitively to the questions on indications to allogeneic HSCT in AML, especially in intermediate risk of older patients and /or of those patients with several comorbidities. Generally allogeneic HSCT is indicated in all MRD positive CR1 patients. The standard transplants are HLA identical sibling and well matched unrelated donors, although several intriguing papers on alternative transplants, in particular haplos, state that they are not only feasible but that the performances are similar to the standard ones. It is an experimental hypothesis to be tested in prospective clinical trials which are approaching. Similarly, for CB transplants, registry or retrospective data suggest a greater graft *versus* leukemia effect compared to unrelated adult donors, especially in MRD positive patients; for this reason, some authors argue that a CB transplant can be offered in a *priority* algorithm in these patients: only a prospective trial will be able to validate such an algorithm choice. About MRD and transplant, differently form acute lymphoblastic leukemia, no sufficient data on long term outcome, complications and feasibility (there is a risk of skipping planned transplants) have yet emerged to enact a MRD driven approach, postponing allogeneic HSCT until MRD negativity has been reached (which is not anyway an easy tool). However, a MRD positive patient should be considered at very high risk of leukemia relapse after allogeneic HSCT and preemptive approaches should be implemented (earlier immunosuppression withdrawal, DLI, pharmacological maintainance when indicated) and it represents an important area of clinical research to be implemented. Relapse after allogeneic HSCT is still the first cause of failure, even in CR1 patients; no gratifying therapies are available for treating the post transplant reappearance of leukemia and results of preemptive approaches targeting MRD or chimerisms, although fascinating, seem to be far from a substancial change of the dismal prognosis of such patients.

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RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS: TREATMENT OPTIONS AND POSSIBLE SEQUENCES

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Summary: Treatment of multiple myeloma (MM) has improved during the past decade with the introduction of proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs). Next generation PIs, such as carfilzomib and ixazomib, and IMIDs such as pomalidomide have been recently introduced into the armamentarium against MM. Drugs with

different mechanism of action, including the histone deacetylase (HDAC) inhibitor panobinostat and the monoclonal antibodies (MoAbs) elotuzumab and daratumumab have further increased treatment options. Although current treatments are highly effective in controlling the disease and producing deep remissions, including molecular remissions, MM eventually relapses. To better estimate the impact of the newer therapies, it is important to understand and define the natural history of myeloma among patients who become refractory to commonly used classes of drugs, such as PIs and IMiDs. Median progression-free survival (PFS) and overall survival (OS) for patients previously exposed to PIs and IMIDs, who received at least one therapy after relapse, were 5.0 and 15.2 months, respectively. For patients who did not receive any further therapy, median OS was 2.1 months.[1] Median OS was 7.5 and 5.1 months among patients double refractory to both PIs and IMIDs and triple/quadruple refractory patients, respectively. This chapter will discuss current treatment options and possible therapy sequencing to optimize outcomes of relapsed/refractory (RR) MM patients.

Table 1. Efficacy and subgroup analyses of novel agent-combinations in phase 3 trials for RRMM.

Trial	Key inclusion criteria	Patients with del5 years %	Overall population			Median PFS by FISH risk		Median PFS by number of prior treatments	
			Overall-response-rate %	Median progression-free-survival (months)	Median Overall Survival (months)	Standard-risk group by FISH (months)	High-risk group by FISH (months)	1 line (months)	>1 lines (months)
ENDEAVOR Kd vs Vd	1-3 prior lines, not PI refractory	52 vs 55	77 vs 63	18.7 vs. 9.4	47.6 vs 40.0	NR vs 10.2	8.8 vs 6	22.1 vs 10.1	14.9 vs 8.4
ASPIRE KRd vs Rd	1-3 prior lines, not Bor and Len refractory	47 vs 53	87.1 vs 66.7	26.3 vs. 17.6	48.3 vs 40.3	29.6 vs 19.5	23 vs 14	29.6 vs 17.6	25.8 vs 16.7
TOURMALINE-MM1 IRd vs Rd	1-3 prior lines, not PI and Len refractory*	53 vs 51	78.3 vs 71.5	20.6 vs 14.7	NR vs NR	20.6 vs 15.6	21.4 vs 9.7	20.6 vs 15.9	§ 2nd line: 17.5 vs 14.1 § 3rd line: NR vs 10.2
CASTOR Dara-Vd vs Vd	≥1 prior line, not primary refractory and PI refractory	47 vs 49	82.9 vs 63.2	NR vs 7.2	NA	NR vs 7	11.2 vs 7.2	NR vs 7.5	§ 2nd line: 10.3 vs 6.5 § 3rd line: 8.8 vs 6.6 § >3 lines: 8.4 vs 5.4
POLLUX Dara-Rd vs Rd	≥1 prior line, not primary refractory and Len refractory	53 vs 51	92.9 vs 76.4	NR vs 18.4	NR vs NR 1-year probability: 92% vs 67%	NR vs 17.1	NR vs 10.2	NR vs 18.4	§ 2nd line: NR vs 11.9 § 3rd line: NR vs NR § >3rd line: NR vs NR
ELOQUENT-2 Elo-Rd vs Rd	1-3 prior lines, not Len refractory	58 vs 56	79 vs 66	19.4 vs 14.9	48.3 vs 39.6	NA	NA	31 vs 19	21 vs 15
PANORAMA 1 PanoVD vs VD	1-3 prior lines, not PI refractory	42 vs 42	60.7 vs 54.6	12.0 vs 8.1	33.6 vs 30.3	NA	NA	NA	NA

Abbreviations: RRMM: Relapsed or Refractory Multiple Myeloma; [1]primary refractory patients included; PI: proteasome inhibitors; KD: carfilzomib-dexamethasone; VD: bortezomib-dexamethasone; KRD: carfilzomib-lenalidomide-dexamethasone; RD: lenalidomide-dexamethasone; IRD: ixazomib-lenalidomide-dexamethasone; Dara-RD: daratumumab- lenalidomide-dexamethasone; Dara-VD: daratumumab-bortezomib-dexamethasone; Elo-RD: elotuzumab-lenalidomide-dexamethasone; PanoVD: panobinostat-bortezomib-dexamethasone; NA: not available; NR: not reached; Bor: bortezomib; Len: lenalidomide; FISH: Fluorescent in situ hybridization; PFS: progression free survival.

Phase III trials for RRMM after 1-3 prior lines: Efficacy and safety results of phase III trials after 1-3 prior lines of therapy are summarized in Table 1 and 2. Carfilzomib is an irreversible, epoxyketone second-generation PI. The ENDEAVOR trial directly compared carfilzomib-dexamethasone (Kd) with bortezomib-dexamethasone (Vd). Median PFS was nearly doubled with carfilzomib (18.7 *vs* 9.4 months, HR 0.53; P<0.0001) and median OS was 47.6 *vs* 40.0 months (HR 0.79, P=0.01). The dosing of carfilzomib was much higher in this trial (56 mg/m^2) than in the combination carfilzomib-lenalidomide-dexamethasone, demonstrating a dose-response relation.[2] The randomized, phase 3 study ASPIRE compared the combination carfilzomib-lenalidomide-dexamethasone (KRd) with Rd. Prior lenalidomide- and bortezomib-treatment was permitted if there was no disease progression on these drugs. Notably, the majority of patients (80.2%) were lenalidomide-naive. The overall response rate (ORR) was significantly higher in the carfilzomib arm compared with the control arm (87.1% *vs* 66.7%; P<0.001), as well as the complete response rate (31.8% *vs* 9.3%). The median PFS was 26.3 *vs* 17.6 months (HR 0.69, P=0.001) and the median OS was 48.3 *vs* 40.3 months (HR 0.79, P=0.01).[3] In pre-planned exploratory subgroup analyses of both trials, the PFS benefit with carfilzomib was observed in all subgroups, including age, cytogenetic risk and number of prior lines of therapy. As expected, the KRd benefit was less evident in patients refractory to bortezomib (22.3 *vs* 19.4 months) and to lenalidomide (11.3 *vs* 9.0 months). This 2-3 month improvement in median PFS is probably due to the acquired drug-resistance. No data are available on double refractory patients. Carfilzomib is associated with a very low incidence of peripheral neuropathy but significant cardiovascular toxicity, fever and infections. Ixazomib is a new, oral boronic acid PI. The randomized, phase 3, TOURMALINE-MM1 study compared ixazomib-lenalidomide-dexamethasone (IRd) with placebo-Rd.[4] Prior lenalidomide- and bortezomib-treatment was permitted if there was no disease progression on these drugs. The ORR was significantly higher in the IRd group (78.3% *vs* 71.5%, P=0.04). Median PFS was 20.6 months with IRd and 14.7 months with placebo-Rd (HR 0.74, P=0.01). Median OS was not reached in either study group. The PFS benefit was consistent in all key pre-specified patient subgroups, including patients with high-risk cytogenetics, International Staging System (ISS) 3, and older than 75 years. The PFS advantage with IRd was greater in patients with 2-3 prior therapies (HR 0.58) and in those with 1 prior therapy without prior transplant (HR 0.60). c-MYC expression was higher in MM tumor samples from patients with 2-3 prior therapies and in those who did not receive transplant. IRd was more active in c-MYC-high patients, providing a potential explanation for the enhanced PFS benefit in patients with 2 to 3 previous lines or in those who did not receive transplant. The most frequent adverse events were thrombocytopenia, rash and gastrointestinal toxicities. MoAbs represent a major step forward in MM treatment. Elotuzumab, an immunostimulatory MoAb targeting signaling lymphocytic activation molecule F7, had no activity as a single-agent but showed activity in combination with lenalidomide and dexamethasone. The randomized, phase 3, ELOQUENT-2 study assessed elotuzumab-lenalidomide-dexamethasone (ERd) *vs* Rd.[5] All patients had received 1-3 previous therapies and had documented disease progression after their most recent therapy. Previous treatment with lenalidomide was permitted if there was no disease progression on this drug. The ORR with ERd was 79%, *versus* 66% with Rd (P<0.001). One-year PFS was 68% in the ERd group and 57% in the control group; 2-year PFS was 41% and 27%, respectively. Median PFS was 19.4 months in ERd patients and 14.9 months in the control group (HR 0.70; P<0.001). Median OS was 48.3 *vs* 39.6 (HR 0.78). The PFS advantage was consistent across key subgroups, including patients ≥65 years, those refractory to the most recent line of therapy, with ISS 3, with previous exposure to bortezomib or IMiDs, with previous stem-cell transplantation, with del(17p), or with a creatinine clearance of less than 60 ml per minute. Common grade 3-4 adverse events were lymphocytopenia, neutropenia, fatigue, and pneumonia. Infusion reactions occurred in 10% of patients and were mainly grade 1-2. Even more promising results have been reported with anti-CD38 antibodies, which already demonstrated activity in monotherapy, with approximately 30% ORR in heavily pre-treated double-refractory patients. Daratumumab, a human IgG MoAb that targets CD38, showed substantial single-agent efficacy and a manageable safety profile in patients with heavily pretreated RRMM. Impressive results have been reported in the randomized, phase 3, POLLUX study comparing daratumumab in combination with Rd (DRd) with Rd.[6] All patients had received at least one previous therapy. Previous treatment with lenalidomide was permitted if there was no disease progression on this drug. A significantly higher ORR was observed in the daratumumab group than in the control group (92.9% vs. 76.4%, P<0.001), as well as a higher ≥ complete response (CR) rate (43.1% vs. 19.2%, P<0.001). One-year PFS was 83.2% in the daratumumab group and 60.1% in the control group; the respective 1-year OS was 92.1% and 86.8%. All pre-specified subgroup analyses of PFS confirmed the benefit of daratumumab, including number of previous lines of therapy and previous exposure to lenalidomide. The PFS advantage with DRd was even present in bortezomib-refractory patients (1-year PFS: 70.8% *vs* 44.4%, HR 0.46, P=0.012). The most common grade 3-4 adverse events during treatment were neutropenia, thrombocytopenia, and anemia. Daratumumab-associated infusion-related reactions occurred in 47.7% of the patients and were mostly of grade 1-2. The randomized phase 3 study CASTOR evaluated daratumumab-bortezomib-dexamethasone (DVd) *versus* Vd.[7] Patients were eligible if they had received at least one previous line of therapy, had at least a partial response to one or more of their previous therapies, and had documented progressive disease during or after the completion of their last regimen. The ORR was 82.9% in the daratumumab group and 63.2% in the control group (P<0.001), with ≥CR rate of 19.2% and 9.0%, respectively (P = 0.001). One-year PFS was 60.7% in the daratumumab group and 26.9% in the control group. Median PFS was not reached in the daratumumab group and was 7.2 months in the control group (HR 0.39; P<0.001). Because of the short follow-up period, median OS was not reached in either treat-

ment group. Prespecified subgroup analyses of PFS confirmed the superiority of daratumumab in all subgroups, including patients who had previously received bortezomib. The most common grade 3-4 adverse events were thrombocytopenia, anemia and neutropenia. Infusion-related reactions were reported in 45.3% of the patients in the daratumumab group, they were mostly grade 1-2, and in 98.2% of these patients, they occurred during the first infusion. Numerous HDACIs have been developed (vorinostat, panobinostat, givinostat, romidepsin and ricolinostat) and all have been tested for the treatment of myeloma patents. However, no significant activity has been reported when used as single-agent. In the phase 3 trial PANORAMA-1, panobinostat-bortezomib-dexamethasone was compared with Vd. RRMM who had received 1-3 previous treatment regimens, but not bortezomib-refractory, were included in this study.[8] There was no significant difference in ORR between the 2 groups (60.7% *vs* 54.6%; P=0.09) but a significantly higher proportion of patients receiving panobinostat achieved a CR or near-CR (27.6% *vs* 15.7%; P < 0001). Median PFS was significantly longer in the panobinostat group (12.0 *vs* 8.1 months; HR, 0.63; P < 0001). The median OS was not significantly different between the two treatment arms (33.6 *vs* 30.4 months). The PFS benefit in the panobinostat-bortezomib-dexamethasone group was more pronounced in patients with high-risk cytogenetics and in those previously exposed to both PIs and IMiDs. As expected, adverse events were more frequent in the panobinostat arm, and 33% of patients had to discontinue treatment *versus* 17% in the bortezomib arm. Most frequent toxicities were diarrhea, thrombocytopenia and fatigue.

Table 2. Main grade ≥3 toxicities of novel agent-combinations in phase 3 trials for RRMM.

Trials	ENDEAVOR (Kd vs Vd)	ASPIRE (KRd vs Rd)	TOURMALINE-MM1 (IRd vs Rd)	CASTOR (Dara-Vd vs Vd)	POLLUX (Dara-Rd vs Rd)	ELOQUENT-2 (Elo-Rd vs Rd)	PANORAMA 1 (PanoVD vs VD)
Haematological adverse events (G≥3)	Anemia 14% vs 10% Neutropenia 2% vs 2% Thrombocytopenia 8% vs 9%	Anemia 18% vs 13% Neutropenia 30% vs 26% Thrombocytopenia 17% vs 12%	Anemia 9% vs 13% Neutropenia 23% vs 24% Thrombocytopenia 19% vs 9%	Anemia 14% vs 16% Neutropenia 13% vs 4% Thrombocytopenia 48% vs 33% Lymphopenia 10% vs 3%	Anemia 12% vs 20% Neutropenia 52% vs 37% Thrombocytopenia 13% vs 13% Lymphopenia 5% vs 4%	Anemia 19% vs 21% Neutropenia 34% vs 44% Thrombocytopenia 19% vs 20% Lymphopenia: 77% vs 49%	Anemia 18% vs 19% Neutropenia 35% vs 11% Thrombocytopenia 67 vs 31% Lymphopenia 53% vs 40%
Non-haematological adverse events (G≥3)	Hypertension 9% vs 5% Dyspnea 7% vs 8% PN 2% vs 9% Cardiac failure 5% vs 2% Fatigue 5% vs 7%	Hypokalemia 9% vs 5% Dyspnea 3% vs 2% Hypertension 4% vs 2% Cardiac failure 4% vs 2% Fatigue 7.7% vs 6.4%	Diarrhea 6% vs 3% Rash 5% vs 2% PN 2% vs 2% Fatigue 4% vs 3%	Pneumonia 8% vs 10% PN 5% vs 7% Infusion related reaction 9% vs NA Fatigue 5% vs 3%	Pneumonia 6% vs 8% Fatigue 6% vs 3% Infusion related reaction 5% vs NA	Fatigue 8% vs 8% Diarrhea 5% vs 4% Infusion related reaction 1% vs NA	Diarrhea 25% vs 8% PN 18% vs 15% Asthenia or Fatigue 24% vs 12%

Abbreviations: RRMM: Relapsed or Refractory Multiple Myeloma; G: grade; KD: carfilzomib-dexamethasone; VD: bortezomib-dexamethasone; KRD: carfilzomib-lenalidomide-dexamethasone; RD: lenalidomide-dexamethasone; IRD: ixazomib-lenalidomide-dexamethasone; Dara-RD: daratumumab- lenalidomide-dexamethasone; Dara-VD: daratumumab-bortezomib-dexamethasone; Elo-RD: elotuzumab-lenalidomide-dexamethasone; PanoVD: panobinostat-bortezomib-dexamethasone; PN: peripheral neuropathy; NA: not available.

Trials for RRMM after more than 3 prior lines: Pomalidomide is a second-generation IMiD with a structure similar to thalidomide and lenalidomide. In the phase 3, MM-003 trial, pomalidomide-dexamethasone (Pd) was formally compared to high-dose dexamethasone in heavily pre-treated patients (median number of prior lines of 5) and most of patients were refractory to both lenalidomide and bortezomib. Pd induced a higher ORR (21% *vs* 3%; p<0.001) and significantly prolonged median PFS (4 *vs* 2 months; p<0.001) and OS (not reached *vs* 8 months, p<0.001).[9] Based on these results, pomalidomide has been approved for the treatment of patients with MM who have received ≥2 prior therapies, including lenalidomide and bortezomib, and experienced disease progression on or within 60 days of completion of the last therapy, becoming the backbone for subsequent trials in this setting. The addition of the alkylating agent cyclophosphamide to Pd doubled the ORR, from roughly 30% to 65%, and prolonged PFS from approximately 4 months to 10 months, without adding significant toxicities.[10] Two trials explored the activity of pomalidomide-bortezomib-dexamethasone (PVd) in patients who had received 1–4 lines of previous therapy.[10] The ORR ranged from 65% to 81%. Neutropenia and thrombocytopenia were the most common grades 3–4 adverse events. These studies provided the basis for an ongoing, randomized, phase 3 study comparing PVd with Vd (MM-007 study). The combination carfilzomib, pomalidomide, dexamethasone was tested in 2 phase I/II trials.[10] Almost all patients were dual-refractory (to both lenalidomide and bortezomib). The ORR rate was 50-58%%, median PFS was 7.2-9.5 months, and median OS was 20.6 months in one study and not reached in the other one. Grade ≥3 non hematological toxicities were congestive heart failure, pulmonary embolisms, renal failures, and pneumonia. A phase I1-2I study explored ixazomib, pomalidomide and dexamethasone in double-refractory patients.[10] The ORR was 62% in 13 evaluable patients. The most frequent grade 3-4 adverse events were neutropenia and infections. The combination of daratumumab-pomalidomide-dexamethasone in patients refractory to bortezomib and lenalidomide but naïve to daratumumab and pomalidomide showed an ORR of 89%, including a CR rate of 42%.[10,11] After a median follow-up of 14 months, median PFS was not reached. Poor results were observed in patients refractory to daratumumab and/or pomalidomide. Numerous new agents and combinations are under clinical evaluation.[11] Isatuximab is a humanized anti-CD38 MoAb that binds to a unique epitope on human CD38, targeting a completely different amino acid sequence than daratumumab. As single-agent, at least a partial response was documented in approximately 30% of patients. In combination with Rd in heavily pretreated patients, including 83% refractory to previous lenalidomide therapy, the ORR was 52%. In combination with carfilzomib-dexamethasone, in heavily pretreated patients, including 63% refractory to previous carfilzomib therapy, the ORR was 80%. %. In combination with pomalidomide-dexamethasone, the ORR was 62%. Infusion-related reactions mainly occurred during the first infusion and were not severe in grade (1-2). The most common treatment emergent toxicities were fatigue and nausea, and the most common drug-related grade 3-4 event was pneumonia. MOR202 is a novel fully human MoAb against CD38 that differs from daratumumab and isatuximab because it does not induce complement-dependent cytotoxicity, which is suspected to be the major contributor to infusion-related reaction. It was tested in a phase 1-2a study alone or in combination with lenalidomide or pomalidomide. Partial response rate was 32% in the MOR202-alone cohort, 71% in combination with lenalidomide and 60% in combination with pomalidomide. Checkpoint inhibitors are another major breakthrough in MM treatment. Preclinical studies provided the scientific rationale for the combination of checkpoint inhibitors (pembrolizumab, anti-PD1, and durvalumab, anti-PD-L1) with IMiDs. In a phase 1, dose escalation trial, pembrolizumab combined with Rd (PembroRd) was tested among RRMM patients; of note, 76% patients were refractory to lenalidomide while 30% were double-refractory. 76% of patients achieved at least a partial response, with a median duration of response of 10 months. Pembrolizumab in combination with pomalidomide-dexamethasone showed an ORR of 56%, including 27% very good partial response (VGPR) or better. For patients with double-refractory disease, the ORR was 55%, including 20% VGPR. Median PFS was 17.4 months. The most frequent adverse events were neutropenia, infections, rash and autoimmune mediated events, including pneumonitis, hypothyroidism, transaminitis, adrenal insufficiency and vitiligo. These data provided the rationale for phase 3 studies. Recently, the FDA has placed a clinical hold on the phase 3 pembrolizumab trials because available data indicate that the risks of pembrolizumab plus pomalidomide or lenalidomide outweigh any potential benefit for patients with MM. An independent data monitoring committee noted an imbalance in deaths among patients with MM assigned to combination therapy involving pembrolizumab and IMiD treatment. Selinexor is an oral selective XPO1 inhibitor. It was assessed as single-agent in 79 patients with disease refractory to bortezomib, carfilzomib, lenalidomide, and pomalidomide (quad-refractory) or refractory to also an anti-CD38 antibody (penta-refractory). The ORR was 21%, including 5% with a VGPR in quad-refractory patients and 20% for penta-refractory patients. Median OS was 9.3 months. In combination with Vd, it induced an ORR of 77%, with ≥VGPR of 27%. In patients refractory to proteasome-inhibitors, the ORR was 58%, indicating that the addition of selinexor restores sensitivity to bortezomib. Selinexor was also combined with carfilzomib-dexamethasone, showing an ORR of 63%, including ≥VGPR of 25%. The corresponding response rates for carfilzomib-refractory patients at the last line of treatment were 64% and 18%. Common adverse events related to selinexor included hematologic and gastrointestinal toxicity. Nelfinavir is an oral human immunodeficiency virus protease inhibitor that has anti-MM activity *in vivo.* It triggers unfolded protein response activation, sensitizes myeloma cells to proteasome inhibitors and overcomes proteasome inhibitor resistance *in vitro*. In combination with Vd in proteasome-inhibitor-refractory patients, the ORR was 65%, including 14.7% VGPR. In patients with double-refractory disease (refractory to proteasome inhibitors and lenalidomide), the ORR was 69%. The most frequent adverse events were

anemia, thrombocytopenia and infections. Venetoclax is a potent, selective, oral BCL-2 inhibitor. As single-agent, the ORR was 21%, including 15% VGPR. Median time to progression was 2.6 months. Most of the objective responses (40%) and better time to progression (6.6 months) were reported in patients with t(11;14), who mostly have a favorable BCL-2 family expression profile (high BCL-2, low BCL-XL, low MCL-1). In combination with Vd, the ORR was 68% and the median time to progression was 8.6 months. The most frequent adverse events included neutropenia, thrombocytopenia, gastrointestinal toxicities and infections.

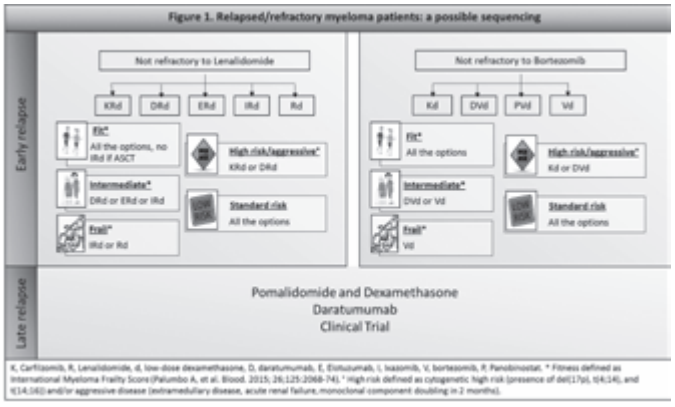

Figure 1.

Sequencing and practical considerations: The choice of the most appropriate regimen for RRMM is a burning question. Multiple factors play a role in treatment selection: host factors (performance status or frailty), disease-specific factors (extramedullary disease, renal dysfunction, cytogenetic risk or refractoriness), time-to-relapse (less than 1 year or more), history of prior therapies and toxicity to previous treatment. Treatment schedule and route of administration may also play a role. Strict inclusion criteria of clinical trials often exclude patients with non-secretory disease, those with renal failure or frail ones. The recent phase 3 trials with carfilzomib-dexamethasone and daratumumab-bortezomib-dexamethasone restricted enrollment to patients who were not refractory to bortezomib. Similarly, the recent phase 3 trials with carfilzomib, elotuzumab, ixazomib and daratumumab using lenalidomide and dexamethasone as a backbone restricted enrollment to patients not refractory to lenalidomide. The large majority of patients in the above trials were lenalidomide-naïve (>80%). This is especially relevant given the increasing use of lenalidomide in newly diagnosed patients ineligible for autologous stem-cell transplant as well as maintenance after autologous stem-cell transplant. Figure 1 shows a possible treatment algorithm to best use available regimens in different patients. Nevertheless, caution is needed when making cross-trial comparisons, due to the differences in patient characteristics and trial design. Briefly, patients not refractory to bortezomib could receive carfilzomib-dexamethasone or bortezomib-dexamethasone as a backbone plus daratumumab or panobinostat. Patients not refractory to lenalidomide could receive lenalidomide-dexamethasone as a backbone plus carfilzomib or elotuzumab or daratumumab or ixazomib. Patients double refractory or with late relapse disease could receive daratumumab as single-agent or pomalidomide-dexamethasone as a backbone plus carfilzomib or daratumumab or cyclophosphamide. Considering the different cut-off value of plasma cells with cytogenetic abnormalities used in each trial, carfilzomib and daratumumab seem to particularly improve outcome in high-risk patients - although none of them completely overcome the poor prognosis - and in patients with aggressive disease. The PFS benefit observed with elotuzumab-Rd and ixazomib-Rd was greater in patients with 2-3 prior therapies and, for ixazomib-Rd, in those with 1 prior therapy without prior transplant. For intermediate-fitness and frail patients, the convenience of a total oral regimen, such as ixazomib-Rd, and the good safety profile of elotuzumab-Rd, have a considerable impact on patient's ability to comply with treatment. Finally, regarding safety, patient co-morbidity should be carefully considered before prescribing a specific drug: cardiac co-morbidities for carfilzomib, pulmonary co-morbidities for daratumumab, gastrointestinal disease for ixazomib and so on. Of note, many current trials are designed for regulatory approval to make new drugs become available in a timely fashion. Unfortunately such trials seldom investigate the ideal sequence of treatment at relapse. In future studies, it will be important to evaluate patient-reported outcome measures of symptoms, quality of life, cost-effectiveness analyses and sequence of therapy to help clinicians to select the optimal treatment for each patient.

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MINIMAL RESIDUAL DISEASE AS A BIOMARKER

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Introduction: Despite the progress achieved in the last decades, the clinical outcome of adult patients with acute myeloid leukemia (AML) still remains largely unsatisfactory, with roughly 40% of young patients and <20% of elderly patients becoming long-term survivors. A possible reason might be that, in spite of the expanding knowledge of the molecular landscape of AML, in the large majority of cases treatment strategy still relies on a "one size fits all" approach. The only exceptions are acute promyelocytic leukemia (APL) and, more recently, FLT3-mutated AML.[1] Not surprisingly, both diseases carry robust predictive biomarkers (*PML-RAR* and *FLT3-ITD*, respectively). Actually, availability of biomarkers is a fundamental step in the attempt to abandon empirical therapy in favor of more rationale stratified approaches. In fact, stratified-based medicine, through the process of biomarker assessment, makes possible that a given patients is associated with a specific therapy.[2] In AML, accessibility to reliable biomarkers is still an unmet need for the large majority of patients. In this context, minimal (or measurable) residual disease (MRD) promises to be a potential, universal biomarker able to guide post-remission choices in AML. In this report we will summarize the current evidence supporting the adoption of the MRD biomarker and discuss pros and contras of such a strategy.

Prognostic and predictive biomarkers: The term biomarker refers to a physical, clinical or biological measurable variable that is associated

with disease outcome. A biomarker is defined prognostic if it informs about cancer outcome (eg, disease recurrence, disease progression, death) independently of treatment received, whereas it is predictive if the treatment effect (experimental compared with control) is different between biomarker-positive and biomarker-negative patients.[2] Biomarkers can be determined at diagnosis, like most of the genetic/molecular abnormalities or after treatment delivery. In the latter case, the purpose of the assessment is to test of the quality of morphological complete remission (mCR), like in case of MRD determination. MRD captures the diversities of the underlying genetic/cytogenetic features of AML and recapitulates patients' heterogeneities regarding drug bioavailability, metabolism and resistance.[3] In fact, even within homogeneous genetic subgroups (eg, *NPM1-Mutated*), the long-term outcome of the patients depends on the clearance of the mutated transcript. Furthermore, at diagnosis some patients may happen to lack specific molecular signatures so that a proper risk profile cannot be codified. Thus, in a comprehensive decision-making process, techniques aimed at determining the quality of mCR may fruitfully complement prognosticators identified at diagnosis.[4]

Stratified medicine: beyond morphological Complete Remission: It is generally accepted that, when a mCR is declared, at least 10^{10} residual leukemic cells still harbor in the patient's bone marrow (BM). In fact, determination of blast percentage by light microscopy is hampered by a limited sensitivity and inter-observer variability. Actually, many studies have demonstrated that, regardless the technique employed, MRD persistence in a condition of mCR, confers a negative prognosis that is comparable to the one associated with morphological persisting leukemia.[5] In spite of this, mCR is still the key parameter to decide whether chemotherapy was effective or not and the post-remission treatment in AML. In this view, to refine the concept of good quality mCR, the revised ELN guidelines for AML treatment have recently introduced the definition of MRD negative mCR.[6] In fact, the authors state that MRD assessment provides a more reliable predictor of outcome than conventional morphology-based CR assessment. A perfect MRD determination assay would precisely quantify residual leukemic cells biologically able to cause relapse within a specific time interval. Multiparameter flow cytometry (MFC) and real-time quantitative PCR (RT-qPCR) are the leading techniques in MRD monitoring, each methodology differing in sensitivity and in the proportion of patients to whom it can be applied. This approaches will be soon implemented by digital PCR, and next-generation sequencing–based technologies.[7] The incorporation of MRD determination into stratified therapy of AML patients will hopefully allow to deliver treatments, intensity of which is proportional to the aggressiveness of AML. This approach will also help avoiding either therapeutic overexposure for patients with a low risk of recurrence or under-treatment for high-risk patients, in whom appropriate treatment intensifications, like allogeneic stem cell transplantation (SCT), should be timely delivered.

Technical issues: quality requirements in MRD detection: Different platforms are available for assessing MRD in AML including traditional light microscopy, fluorescence *in situ* hybridization, cytogenetics, MFC, RT-qPCR, and next-generation sequencing (NGS), all different in sensitivity, specificity, and phase of development (extensively reviewed in Ossenkppele *et al.*[7]). Reverse Transcriptase-quantitative PCR (RT-qPCR) is highly reproducible between laboratories, turnaround time is rapid and risk of contamination is substantially reduced. Furthermore, the capacity to give a quantitative estimation of the residual transcripts and the standardization of housekeeping genes enumeration to avoid false negative results makes this method the most validated for quantitative monitoring of MRD. A major drawback of RT-qPCR is its applicability only to those patients with molecular targets that are specific and stable over the treatment course, accounting for roughly 50% of young AML cases, and the possible contamination by not-viable cells carrying the same molecular target. Common targets of RT-qPCR are fusion genes generated by balanced chromosomal rearrangements [eg, *PML-RARA* t(15;17), *RUNX1-RUNX1T1* t(8;21), *CBFB-MYH11* (inv(16) t(16;16), t(11q23) *MLL* fusions, *DEK-CAN(NUP214)* t(6;9)], insertions/duplications (eg, *NPM1*, FLT3-ITD, MLL-PTD), point mutations (eg, CEBPA, IDH1/2, KIT, RAS, etc). Among these targets, CBF-AML and NPM1 have received an extensive clinical validation and today represent technical platforms suitable for application in clinical practice.[3] Furthermore, the quantitative determination of Wilms tumor gene (WT1) expression, which is generally overexpressed in AML BM and peripheral blood (PB), has been extensively evaluated. A major factor affecting assay sensitivity and clinical utility is that expression of *WT1* is not leukemia-specific, thus limiting the capacity to distinguish MRD from normal background, especially in BM. MRD monitoring by MFC relies on the presence on leukemic cells of a combination of antigens and/or flow cytometric physical abnormalities that are absent or very infrequent in normal BM (eg, cross-lineage expression, over-expression, reduced or absent expression and asynchronous expression). Detection of leukemia-associated immunophenotypes or detection of different-from-normal phenotypic patterns represent two complementary strategy of analysis.[7] The growing interest surrounding MFC is due to its wide applicability (>90% of AML), quickness, specificity and ability to distinguish viable cells from BM debris and dead cells. Furthermore, the commercialization of devices equipped with multiple lasers allows multiple color assays to be implemented thus favoring increment of sensitivity that, at the present time, can be reasonably placed in between 10^{-3} and 10^{-5}. An additional advantage of using policromatic panels of at least 8 colors consists in a significant attenuation of concerns of phenotypic shifts that can be observed upon recurrence. Despite its clinical value as a biomarker to inform therapy is difficult to ignore, the mayor drawback of MFC-MRD is that it has been generated by few laboratories with a specific and robust expertise.[7] A progress in standardization and/or harmonization is warranted, and scientific societies, like European LeukemiaNet, are trying to endorse common approaches to define time-points, thresholds, panels and results reporting of MFC-MRD. Digital PCR (dPCR) is a refinement of conventional PCR methods that can be used to directly quantify and clonally amplify nucleic acids and allows a more reliable collection and sensitive measurement of nucleic acid amounts with no need of a standard curve. It has been shown to be a reliable tool for MRD assessment in lymphoid malignancies and it is also applicable for a large variety of NPM1 mutation. NGS gives the opportunity to study large number of somatic mutations in one single experiment. This feature appears to be particularly useful in AML, where the wide intraclonal heterogeneity often makes the leukemic clone a moving target. Despite some reports have contributed to depict the clonal architecture of AML and clarify the differential response of each mutation to chemotherapy, standardization is currently lacking and the sensitivity level, currently estimated about 10^{-3}, cannot compete with other MRD measurements techniques.

Discordant cases in MRD: One of the recurrent criticisms raised against the role of MRD as a biomarker is the occurrence of discordant cases, namely MRD positivity in prolonged CR or relapse in a status of MRD negativity. The former situation ("false positive") can be explained by assuming that the MRD test detects leukemic markers on normal cells (eg, cells with monocytic mature or stem cells phenotype), pre-leukemic cells or leukemic cells that are not able to generate clonal progenies (e.g. persistence of a leukemic clone carrying a non-fondant mutation). Another possible explanation, at least for patients undergoing allogeneic SCT, is the immunological control operated by the graft-versus-leukemia effect. For relapse occurring in MRD negative ("false negative") patients, many explanations may be advocated. On a technical ground, lack of sensitivity, inadequate sampling and phenotypic shifts are the most commonly recognized explanations. On a biological ground, we should consider differences in time of onset of the leukemic re-growth. More recently, a lot of attention has been dedicated to the role of persistent residual leukemic stem cells (LCSs) in promoting relapse in cases where MRD burden is reduced below the limit of detection.[7] In fact, LSCs may be detected in the BM of many patients in mCR, including those apparently MRD negative. LSCs usually reside in the $CD34^+CD38^-$cells fraction, at least in $CD34^+$AML cases, and carries a specific array of phenotype abnormalities. When combined, measurement of MRD and residual LSCs leads to a further refinement of prognostication.[7]

Clinical application of MRD: risk adapted strategy and pre-transplant prognostic value: Definitive inclusion of MRD in the decision-making process requires that its benefit as a biomarker is proven not only in retrospective cohorts but also in prospective controlled studies. Whatever the techniques used, several retrospective reports confirmed the prognostic role of MRD assessment; on the other hand, few studies have prospectively addressed this issue.[7] In the pediatric protocol AML02, risk-categories were recognized based on the baseline cytogenetic/genetic profile and the level of FCM-MRD after the first cycle of chemotherapy. Treatment intensification by adding gemtuzumab ozogamicin was decided for patients in whom high levels of MRD were measured. The study showed a superior clinical outcome for the recruited patients as

compared to those treated within the previous not risk-adapted trials of the same institution.[8] In the GIMEMA AML1310 trial, adult patients with intermediate-risk AML (intermediate karyotype or FLT3-TKD positive or c-kit mutated CBF positive) received autologous or allogeneic SCT depending on the level of MRD (with a threshold set at the level of 0.035%), measured by flow cytometry after consolidation therapy. Overall survival and disease free survival at 24 months were 78.6% and 61.4% in MRD positive and 69.8% and 66.6% in MRD negative patients. These results suggest that in the intermediate risk-category, allogeneic SCT can be avoided if MRD is not detectable; if MRD is positive, allogeneic SCT can prolong OS and DFS to equalize those of the MRD negative category.[9] Further retrospective observations in two series of CBF-AML and NPM1 mutated AML suggest that patients with MRD persistence above a given threshold of log-reduction may benefit from allogeneic SCT. The same benefit is not observed in patients achieving a significant reduction of MRD levels. Despite this observation, there is not yet a general consensus on the role of MRD as a biomarker dictating the choice between allogeneic SCT and conventional post-consolidation therapy (autologous SCT and/or chemotherapy). In fact, several studies have demonstrated that the pre-transplant MRD status is prognostically informative for patients undergoing myeloablative or nonmyeloablative allogeneic SCT in first CR. Compared to MRD negative patients, subjects with a MRD positive mCR have a 3-year cumulative incidence of relapse and overall survival of 60% to >70% and 20-30%, respectively, with MRD being the prevalent risk factor for adverse outcome. Whether the amount of MRD influences post-transplant outcomes remains controversial. Retrospective analyses from a larger cohort of adults with AML found no statistically significant differences in terms of outcome between patients with lower and those with higher amounts of MRD. In contrast, two smaller studies that included pediatric and adult patients suggested a quantitative effect of MRD; patients with "high-positive MRD" experienced worse outcomes than those with "low-positive" MRD, whereas patients with "low-positive" MRD did worse than those without MRD (extensively reviewed in Buccisano & Walter[10]).

Summary and Conclusions: For the first time, the revised ELN guidelines for AML treatment have introduced the definition of MRD negative mCR. In fact, technical progress has produced molecular and phenotypical assays with reasonably high sensitivity and specificity providing a more reliable predictor of outcome than conventional morphology-based CR assessment. This defines MRD as a prognostic biomarker, in that it is a measurable biological variable that allows to discriminate subgroups of patients with different prognosis and possibly suitable for different treatment allocation to enhance their clinical outcome. The final goal should be exploiting MRD as a surrogate end point for clinical trials. For regulatory purposes, becoming a surrogate end-point implies that MRD is rigorously proved to be a valid surrogate for survival. At the present time, with the only exception of APL, there are no convincing analyses showing that MRD negative CR correlates with survival for different AML populations, different subtypes of AML, different disease states or after different therapies. This purpose will be accomplished only when prospective randomized trials evaluating MRD driven therapy-interventions combined with a survival end point or co-end point within homogeneous subgroups of non-APL AML, will be completed.

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IRON DISORDERS: FROM RESEARCH TO NOVEL THERAPEUTIC STRATEGIES

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Introduction: Advances in basic research have revolutionized our knowledge of the regulation of iron metabolism. It all started with the identification of genes mutated in hereditary hemochromatosis at the turn of the millennium, including the liver hormone peptide hepcidin, which is the central regulator of systemic iron homeostasis. Hepcidin cooperates with the intracellular IRE-IRP system to provide all body cells with the correct iron balance in order to avoid both iron deficiency and iron excess and to adjust iron absorption and recycling according to the body needs. Thanks to the study of hepcidin regulation in animal models the mechanisms of body iron regulation are almost completely elucidated, paving the way to the development of novel and targeted therapeutic strategies for genetic and acquired iron disorders. Traditional iron disorders are iron deficiency and iron overload; iron misdistribution occurs in other cases, the most important being inflammatory and neurodegenerative disorders, the latter not discussed here. A classification of iron disorders based on the hepcidin levels is proposed in **Table 1**.

Disorders with low hepcidin: The study of hereditary hemochromatosis was crucial to our understanding of hepcidin function and contributed advances relevant also for other disorders of iron metabolism. Hemochromatosis is due to molecular defects in the hepcidin-ferroportin axis: hepcidin deficiency, leading to excessive iron import and recycling through ferroportin, may cause iron overload and organ failure. The recessive forms of hemochromatosis are all due to mutations in genes (*HFE, HJV, HAMP, TFR2*) involved in hepcidin upregulation[1]. *HAMP* (hepcidin) and *HJV* (hemojuvelin) have a key role, since their inactivation causes the severe phenotype of juvenile hemochromatosis, while mutations of *HFE* and *TFR2* are less relevant as they lead to less severe iron overload. In all cases, except in the rare mutations that directly affect hepcidin, hepcidin activation through the BMP/SMAD pathway is defective. Dominant hemochromatosis affects the hepcidin receptor ferroportin leading to "ferroportin disease". Mutations that decrease the surface expression of ferroportin or its ability to export iron reduce iron recycling and cause atypical iron overload and macrophage iron retention. Mutations in the hepcidin binding site of ferroportin, the amino acid 326, result in hepcidin resistance and parenchymal iron overload, as in classic hemochromatosis, because of excessive iron export to plasma through ferroportin, not degraded by hepcidin (**Table 1**). Hepcidin is physiologically low/absent in the common acquired forms of iron deficiency to compensate the lack of iron. Pathological hepcidin insufficiency explains iron overload in the so-called "iron-loading anemias", as in non-transfusion-dependent thalassemia syndromes (**Table 1**), disorders characterized by expanded, ineffective erythropoiesis and elevated iron stores. In these patients hepcidin is excessively inhibited by expanded erythropoiesis, which is not limited by blood transfusions. Erythroblasts stimulated by erythropoietin release erythroferrone[2] and possibly other mediators (GDF15, PDGF-BB) to signal their iron needs to the liver, providing a false signal of iron deficiency. Iron absorption is increased together with the availability of plasma iron. However, because of ineffective erythropoiesis, iron incorrectly utilized accumulates, leading to liver and other organs iron overload even in the absence of blood transfusions.

Disorders with high hepcidin: Iron-refractory iron-deficiency anemia (IRIDA) is a rare recessive disorder with a phenotype opposite to hemochromatosis characterized by iron deficiency in the presence of inappropriately high hepcidin production. IRIDA mutations affect the hepcidin protease inhibitor *TMPRSS6*. Patients are anemic since first infancy, with microcytic and hypochromic red cells, low serum iron and normal/high hepcidin levels that cause refractoriness to oral iron treatment, hence the name IRIDA[3]. Genetic iron disorders strengthen the fundamental role of the hepcidin-ferroportin axis in the regulation of systemic iron balance[1]. Hepcidin is also an essential mediator of ane-

mia of chronic disease (ACD) or anemia of inflammation (AI), a multifactorial anemia commonly associated with chronic infections/inflammation/cancer and other chronic conditions that are frequent in hospitalized patients. In AI increased proinflammatory cytokines and hepcidin, both host defense mechanisms, induce macrophage iron retention and restrict iron for erythropoiesis[4]. The resulting hypoferremia is taken as an adaptive mechanism that contrasts the growth of extracellular pathogens. This effect underlines the defensin-like "antimicrobial" function of hepcidin. Anemia of inflammation is the most common example of normal total body iron with altered distribution: iron reduction in plasma and erythroid cells and iron abundance in stores.

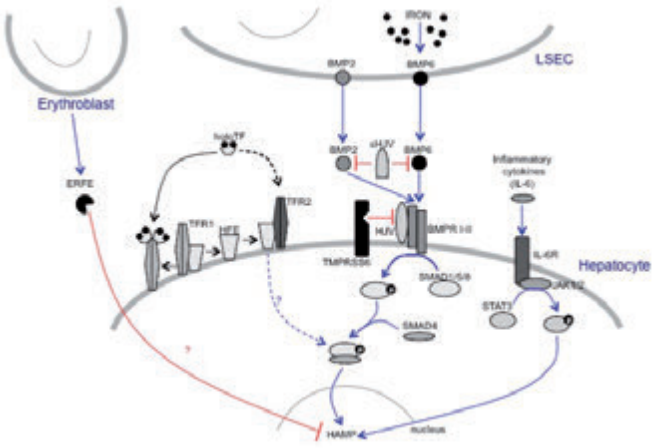

Figure 1. Schematic representation of hepcidin regulation, the scheme shows the cell crosstalk that regulates hepcidin in the liver. Liver sinusoidal endothelial cells (LSEC) produce bone morphogenetic proteins (BMP), especially BMP6 in response to iron; hepatocytes BMP receptors (BMPR I and II) and the coreceptor Hemojuvelin (HJV) bind the ligand. After BMP binding SMAD 1/5/8 are phosphorylated (P), make a complex with SMAD4 and the complex translocates to the nucleus. Hepcidin (HAMP) is also activated by TFR2-HFE through an unknown pathway (?) that impacts on the SMAD pathway. HFE binds both to transferrin receptor TFR1 and TFR2 according to the levels of diferric-transferrin (Holo-Tf). Hepcidin is also activated by inflammatory cytokines (IL-6) through IL-6 receptor and JAK1/2 and STAT3. Inhibition of the BMP pathway requires TMPRSS6, which cleaves HJV in the liver. Also erythroferrone (ERFE) released from erythroblasts inhibits hepcidin with an unknown mechanism (?). Soluble HJV blocking BMPs is an additional mechanism of hepcidin inhibition. Blue lines indicate activation; red lines indicate repression.

Hepcidin regulation: Animal models that recapitulate genetic iron disorders have been essential to our understanding of hepcidin regulation especially on the role of the BMP/SMAD signaling pathway, as summarized in **Figure 1**. The activation of the SMAD pathway in hepatocytes occurs when the ligand (BMP6 when iron increases in tissues) and BMP2 (likely in basal conditions) binds the constitutively active BMP type II receptors. These receptors phosphorylate BMP type I receptors to start SMAD protein activation and nuclear translocation. It has been proposed that hemojuvelin (HJV), which also binds BMPs, favors the transfer of BMP inside the cell, where the binding HJV/BMP is competed by type I BMPR. Interestingly this signaling requires a crosstalk among liver cells, especially the sinusoidal endothelial cells, secreting BMP2 and BMP6 and the hepatocytes, which produce hepcidin (**Figure 1**). Although TFR2 and HFE form a complex with HJV and modulate the activity of the BMP/SMAD signaling the molecular mechanisms of their hepcidin activation remain unclear. A very recent finding is the discovery of the autonomous role of the hepcidin ferroportin-axis in some organs, as the kidney and the heart[5], that might explain the occurrence of iron deficiency independently of anemia, an issue that has important clinical implications, *e.g.* in heart failure. In inflammation hepcidin is activated by proinflammatory cytokines, especially by IL-6, a powerful stimulator of hepcidin through JAK1/2 and STAT3 signaling. For a full hepcidin activation this pathway requires the cooperation and integrity of the BMP pathway. The BMP/SMAD signaling pathway also receives inhibitory signals when hepcidin must be suppressed, as it occurs in iron deficiency, hypoxia and erythropoiesis expansion. In the liver an essential inhibitory role is exerted by the transmembrane protease TMPRSS6/matriptase 2, which cleaves the BMP coreceptor hemojuvelin from plasma membrane [1], suppressing activating signaling. This effect accounts for the BMP/SMAD pathway over-activation and the inappropriately high hepcidin levels in mutant TMPRSS6 in IRIDA. Among the different candidates proposed to inhibit hepcidin and allow iron absorption in accordance with the erythropoietic needs (the "erythroid regulators")[6] erythroferrone (ERFE) appears to fulfill all requirement for this function. ERFE is a member of C1q-Tumor-necrosis factor (TNF)-alpha related family, previously reported, as Fam132b, to have a role in lipid regulation. It is expressed in multiple tissues; however, erythropoietin administration in mice increases its expression only in erythroid cells of bone marrow and spleen. ERFE downregulates hepcidin expression after hemorrhage, phlebotomy and after erythropoietin administration. ERFE is active also in hypoxia where most hepcidin suppression is erythropoiesis-dependent. The inhibitory mechanism of hepcidin by ERFE is unknown, but the effect of ERFE requires the attenuation of the BMP signaling[6]. Another proposed player in hepcidin inhibition in hypoxia is Platelet-derived growth factor-BB (PDGF-BB), which is expressed by several cell types. PDGF-BB suppresses hepcidin both *in vitro* and *in vivo* in human volunteers, by down regulating the transcription factors CREB and CREB-H. Some hormones as testosterone inhibit hepcidin, while among drugs, the mTOR inhibitor rapamycin is an activator of the pathway.

Table 1. Classification of systemic iron disorders according to hepcidin levels.

Inappropriately low hepcidin			
Genetic disorders	Gene	Phenotype	Potential treatment
Hemochromatosis			
Type 1	*HFE*	Iron overload	hepcidin agonists
Type 2A	*HJV*	Iron overload (severe)	hepcidin agonists
Type 2B	*HAMP*	Iron overload (severe)	hepcidin mimics
Type 3	*TFR2*	Iron overload	hepcidin agonists
Ferroportin disease			
Type 4A	*FPN*	Macrophage iron overload	?
Type 4B	*FPN*	Hepcidin resistance	anti-FPN Ab
Acquired disorders			
Iron loading anemias			
NTD beta-thalassemia		Anemia, iron overload	hepcidin agonists
Dyserythropoietic anemias		Anemia, iron overload	hepcidin agonists
Sideroblastic anemias		Anemia, iron overload	hepcidin agonists
Inappropriately high hepcidin			
Genetic disorders	Gene	Phenotype	Potential treatment
IRIDA	*TMPRSS6*	Iron deficiency anemia	hepcidin antagonists
Acquired disorders			
Anemia of inflammation in:			
Chronic infections		Iron restricted erythropoiesis	hepcidin antagonists
Chronic renal failure		Reduced hepcidin excretion + Iron restricted erythropoiesis	hepcidin antagonists
Cancer		Iron restricted erythropoiesis+ other causes	hepcidin antagonists

NTD = Non transfusion dependent
Ab = antibodies
? = unknown

Other recently identified functions/players in iron metabolism: IRP1 and IRP2 are longtime known to regulate intracellular iron proteins. Recent findings indicate that their function is not redundant and that they have specific targets: IRP1 regulates HiF2-alpha, the major kidney sensor of hypoxia and stimulator of erythropoietin synthesis, while IRP2 regulates L-ferritin production in the liver. Conditional deletions of these regulators in specific tissues are revealing different functions of the two proteins. Other molecules with a role in iron regulation are emerging, as erythroid TFR2 and NCOA4. TFR2 is a partner of erythropoietin receptor (EPOR) in erythropoiesis. It associates with EPOR in the endoplasmic reticulum and contributes to deliver the receptor to the cell membrane, where they remain associated. Specific deletion of Tfr2 in mouse bone marrow results in erythrocytosis. This appears to result

from erythroid cells being hypersensitive to erythropoietin, as demonstrated by normal erythropoietin levels but increased expression of its target genes. Being expressed in both hepatocytes and erythroid cells, TFR2, which is stabilized on the cell surface by diferric transferrin, can sense circulating iron simultaneously in two different sites and thereby coordinates erythropoiesis and hepcidin response[6]. The Nuclear Receptor Coactivator 4 (NCOA4) is a new player in iron metabolism that promotes the autophagy of the iron storage ferritin, a process known as "ferritinophagy" which facilitates iron recovery from stores. Beside being a cargo receptor for ferritin, NCOA4 has multiple, incompletely understood functions, that include the control of DNA replication origins and the activation of androgen transcription; it is also involved in cancer, especially thyroid cancer. *Ncoa4-ko* mice are characterized by ferritin and iron accumulation in several organs, especially within macrophages. Iron retention reduces macrophage iron recycling and its availability for erythropoiesis causing mild microcytic anemia. Interestingly the nuclear function of NCOA4 to control DNA replication origins is distinct from its cytosolic function of ferritin cargo, which is essential in iron deficiency[7].

Table 2. Potential novel targeted therapeutic approaches for disorders with altered hepcidin.

To increase hepcidin			
Compounds	Tested in	Mechanism	Disorders
Hepcidin mimics			
Minihepcidin*	*Hamp-/-*, *Hbb*$^{th3/+}$	Iron redistribution Iron restriction	Hemochromatosis Beta-thalassemia
Transferrin infusions			
Apo- or	*Hbb*$^{th3/+}$	Iron restriction	Beta-thalassemia
Holo-transferrin	*Hbb*$^{th3/th3}$	Iron restriction	Beta-thalassemia
Tmprss6 inhibition			
Genetic deletion	*Hbb*$^{th3/+}$ *Hfe-/-*	Iron restriction Iron depletion	Beta-thalassemia Hemochromatosis
Tmprss6 siRNA*	*Hbb*$^{th3/+}$ *Hfe-/-*	Iron restriction Iron depletion	Beta-thalassemia Hemochromatosis
Tmprss6 ASO*	*Hbb*$^{th3/+}$ *Hfe-/-*	Iron restriction Iron depletion	Beta-thalassemia Hemochromatosis
Commercially available drugs			
Rapamycin	*Hbb*$^{th3/+}$	mTOR inhibition	?
To decrease hepcidin	Tested in	Mechanism	Disorders
Suppression of hepcidin synthesis			
Anti-cytokines or their receptors	Patients	Decreased IL-6 pathway	AI
Anti IL-6 receptor Ab	Patients, *Hbb*$^{th3/+}$	Decreased IL-6 pathway	AI
Erythropoiesis-stimulating agents	Patients	Released ERFE (?)	AI
Gene silencing of hepcidin	*Hbb*$^{th3/+}$	Decreased hepcidin synthesis	AI
Hepcidin neutralization			
Anti-hepcidin MoAb	*Hbb*$^{th3/+}$	Block of hepcidin function	AI
Spiegelmers (NOX)H94)	Primates Human volunteers	Block of hepcidin function	AI
Anticalins (PRS-080)	Human volunteers CKD patients	Block of hepcidin function	AI
Interfering with hepcidin effect on FPN			
Anti-FPN Ab	*Hbb*$^{th3/+}$	Block of FPN function	AI

Murine models are in italics: *Hbb*$^{th3/+}$ and *Hbb*$^{th3/th3}$ are models of Non-transfusion-dependent thalassemia
*Also in combination therapy with deferiprone.
MoAb = monoclonal antibodies; CKD = chronic kidney disease; FPN = ferroportin; AI = anemia of inflammation; ERFE = erythroferrone

Targeting the hepcidin pathway for therapy: Manipulation of the hepcidin pathway is a novel attractive therapeutic strategy for iron disorders. In theory hepcidin agonists are useful in cases of low hepcidin, as hemochromatosis and beta-thalassemia, while hepcidin antagonists are indicated in disorders with high hepcidin, as IRIDA and anemia of inflammation[9]. Agonists of hepcidin that have been tested in preclinical studies are reported in **Table 2**. Minihepcidins, short synthetic peptides corresponding to the 9 N-terminal residues of the mature peptide administered to hepcidin-deficient mice prevent iron accumulation in young animals and induce iron redistribution towards macrophages in adult mice. However, considering that in hemochromatosis phlebotomy-based treatment is simple, cost effective and usually well-accepted, hepcidin agonists are under development for the treatment of thalassemia. Indeed minihepcidins decrease iron overload with a partial correction of anemia in the beta-thalassemia *Hbb*$^{th3/+}$ mice[9]. Activation of the SMAD pathway through BMP administration is unfeasible because of the side effect of ossification induced by BMPs. On the contrary silencing the hepcidin inhibitor TMPRSS6, both by small interfering RNA (siRNA) and allele-specific oligonucleotide (ASO) is an interesting approach. Both techniques increase hepcidin and decrease iron overload in murine models of *Hfe* hemochromatosis; most importantly both increase red cell number decreasing spleen size and iron overload in beta-thalassemia mice models. A similar effect is obtained by infusions of exogenous transferrin. Limiting iron to erythropoiesis in beta-thalassemia is beneficial in multiple ways, because it reduces the excess alpha chains, hemichrome formation, ROS generation and thus erythroid cell oxidative damage, apoptosis and death[9]. Since heme is a strong regulator of translation, low heme synthesis likely impairs alpha globin translation, provokes chain imbalance, decreases the oxidative damage and, limiting ineffective erythropoiesis, it ameliorates erythroblast maturation. Also in combination with iron chelators (deferiprone) both minihepcidins and TMPRSS inhibitors maintain their activity, a relevant observation since some of the proposed drugs are now entering phase I/II clinical trials. Inappropriately high hepcidin levels may be in theory antagonized by different compounds in IRIDA as well as in anemia of chronic disease. Anti-HJV antibodies have proven effective in animal models of IRIDA. However, IRIDA is rare, anemia is not severe and is usually responsive, at least partially, to intravenous iron. Thus anemia of inflammation becomes the natural target of treatment with hepcidin antagonists. These compounds affect the pathway that activates hepcidin at different levels and with different mechanisms (**Table 2**). Some compounds such as anti-cytokines drugs are commercially available, but their effect on hepcidin cannot be distinguished from that on the disease control. Others are being or have already been tested in phase I/II clinical trials. However, correcting anemia of inflammation is a cumbersome process, since increased hepcidin is only one component within the multifactorial pathogenesis of anemia, with reduced erythropoietin production and low erythroblast response being other important determinants. Correcting hepcidin without acting on the other cytokines involved may achieve only partial benefits. However, several attempts are ongoing especially in anemia of kidney failure and anemia of cancer. Modulation of the local hepcidin/ferroportin has been proposed in some types of cancer to reduce intracellular iron and to control proliferation. Our unpublished results indicate that deleting TFR2 in erythroid cells allows increasing erythropoietin sensitivity in thalassemia, while restricting iron availability. Theoretically modulating the activity of NCOA4 might be alternative to the modulation of ferroportin. Understanding the regulation of systemic iron homeostasis has provided a wide range of novel therapeutic targets worth exploring in clinical setting. Increasing our knowledge of iron regulation may offer in the future other important tools to be exploited in conditions where iron homeostasis is deranged.

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USE OF PROGNOSTIC MODELS IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Philadelphia-negative Myeloproliferative neoplasms (MPNs) are a group of clonal disorders characterized by myeloproliferation expressing with variable degree of bone marrow hypercellularity and fibrosis, and clinically with predisposition to thrombosis, hemorrhage, and clonal evolution. MPN clinical course varies from one to over 30 years (yrs) and may evolve from asymptomatic into progressive BM failure, symptomatic splenomegaly and acute leukemia in 3-20% of cases. Several prognostic scoring systems have been proposed in MPNs in the last years, that are focused on the main disease-related complications, including vascular complications in polycythemia vera (PV) and essential thrombocythemia (ET), evolution into post-PV/post-ET myelofibrosis or progression to acute leukemia (AL) and premature death in myelofibrosis (MF).

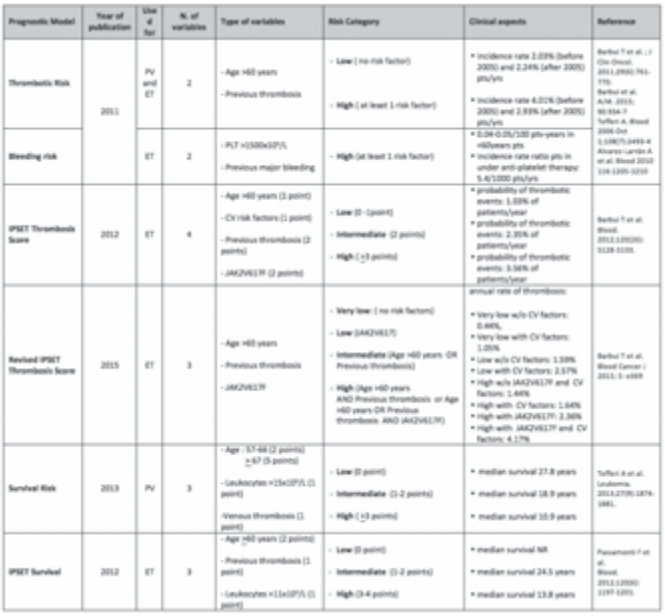
Risk stratification in Polycythemia Vera and Essential Thrombocythemia: In general, life expectancy in polycythemia vera (PV) and essential thrombocythemia (ET) is shorter compared with control population matched for age and gender. Most recent survival data from Mayo Clinic in 559 patients showed a median survivals of approximately 20 years for ET and 14 years for PV(1). Risk factors for overall survival in ET and PV included advanced age, leukocytosis and thrombosis, in particular those in venous district for PV patients. Abnormal karyotype at diagnosis, documented in about 12% of the patients, is an additional risk factor associated to a median survival ranging from 6 to 29 years. The same holds true for somatic mutations shown to affect outcome also in other myeloid malignancies. Adverse somatic mutations, harbored in about 15% of patients, were associated with inferior survival in both PV and ET (median overall survival of 7.7 and 9.0 yrs, respectively) and include *ASXL1*, *SRSF2*, and *IDH2* in PV and *SH2B3*, *SF3B1*, *U2AF1*, *TP53*, *IDH2*, and *EZH2* in ET. These prognostically detrimental mutations predicted also leukemic and fibrotic progression; however, the number of mutations, prognostically unfavorable in myelofibrosis, mutational status of driver mutations (*JAK2*/*CALR*/*MPL*) or their allele burden did not provide additional prognostic information in either ET or PV. The primary objective of treatment in PV and ET is to prevent thromboembolic or hemorrhagic complications occurring in about 15% of cases during disease course. In this regards, a prognostic scoring system validated in recent years is based on patient-oriented characteristics including age more than 60 yrs and history of thrombosis. These 2 simple variables allow to categorize patients into 2 risk groups: low risk (no risk factors) and high risk (1 or 2 risk factors)(2). In addition, according to the literature, from 5% to 30% of patients with ET may develop bleeding disorders as consequence of an acquired von Willebrand syndrome (AVWS) usually observed with extreme thrombocytosis (platelets count greater than 1000-1500×10^9/L). In this cases, in line with the current guidelines, the use of aspirin requires caution. Measurement of VWF pro-peptide level or ristocetin cofactor activity may also be useful in AVWS diagnosis. Nevertheless, the true prevalence of AVWS is difficult to establish due to its low detection rate and frequent misdiagnoses. Other risk factors have been more recently associated with an increased risk of developing major thrombotic events, including hypertension and previous arterial events, for arterial thrombosis in PV and cardiovascular (CV) risk factors, leukocytosis, and presence of *JAK2*V617F in ET, and for venous thrombosis older age and venous events in PV or male gender in ET. A comprehensive assessment and control of additional CV risk factors including smoking, hypertension, diabetes, obesity and dyslipidemic syndrome is becomning increasingly important in thrombotic disease prevention. The International Working Group for MPN Research and Treatment (IWG-MRT) developed the IPSET-thrombosis score, a new prognostic model for thrombotic risk stratification in ET patients recently revised, prone to quantify the individual and combined risk contribution of CV risk factors and JAK2 mutation in both conventionally defined low- and high-risk ET. Revised IPSET-score is based on three risk factors including age greater than 60 years, history of thrombosis and JAK2VF mutation. This score delineated four risk stratification categories: very-low risk (age ≤60 years, no thrombosis history, and absence of *JAK2* mutation); low risk (age ≤60 years, no thrombosis history, and presence of *JAK2* mutation); intermediate risk (age >60 years, no thrombosis history, and absence of *JAK2* mutation); and high risk (age >60 years and/or thrombosis history, and presence of *JAK2* mutation). Such distinction is practically relevant in the very low risk disease in absence of CV risk factors in which low dose of aspirin could be not mandatory. More recently it has been also demonstrated that the prognostic ability of IPSET score was not modified by CALR mutations, suggesting that this score can be used in any patients with a diagnosis of ET. Despite aimed are improving thromboembolic risk stratification, this score is not extensively used for decision making in clinical practice of ET patients. The main prognostic scores in PV and ET are listed in Table 1. Progression to myelofibrosis represents a natural evolution of PV and ET; less than 10% of PV and 5% of ET patients evolve into MF after their first decade from diagnosis. Transformation to MF occurs late during the course of the disease, overall median time to progression from diagnosis is approximately 8-20 years in PV and 7-16 years in ET. A longer time from diagnosis strongly impacts on MF transformation rate: in PV patients a disease duration over ten years reached an hazard ratio of 15. Homozygosity for *JAK2*V617F is usually associated with MF development, supporting the idea that accumulation of mutated alleles usually accompanies transition to myelofibrosis. In a study with 320 PV patients a mutant allele burden greater than 50% was identified as an independent risk factor for postPV-MF (PPV-MF) progression. Retrospective study identified additional risk factors for secondary-MF which include: thrombocytosis, splenomegaly, evidence of BM fibrosis at diagnosis and a diagnosis of 'masked PV' in PV patients and older age, anemia and bone marrow hypercellularity with increased reticulin fibers in ET. At this regards, Barbui et al(3), reported that differences in cumulative incidence rates of transformation in ET are largely due to incorrect diagnosis, between true-ET and prefibrotic myelofibrosis (Pre-PMF). A closer adherence to the recently revised criteria for pre-PMF proposed by WHO will help to prevent these discrepancies. As regards *CALR* variant subtypes, it has been found a significantly higher risk of myelofibrotic transformation in ET patients carrying type1/type1-like than in those carrying type2/type2-like *CALR* mutations. Leukemic transformation rates at 20 years are estimated at less than 5% for ET and 10% for PV. Risk factors for leukemic transformation have been identified in PV including advanced age, leukocytosis, and abnormal karyotype; in ET, leukocytosis ≥15×10^9/l), extreme thrombocytosis (≥1000×10^9/l), anemia, older age (≥60 years), reticulin grading, and bone marrow cellularity. In particular, combination of anemia (12g/dl in females or 13.5 g/dl in males) and thrombocytosis (≥1000×10^9/l) defined a high-risk group of ET patients with a rate of acute leukemia transformation of 6.5% versus 0.4% in those without risk factors. Information regarding the prognostic significance of driver mutations and/or the allele burden in leukemic evolution are not consistent. In both PV and ET statistical association has been found between *JAK2* mutation or the allele burden and the time of evolution to acute leukemia or overall survival has been described. Overall, *JAK2*V617F is not considered a prerequisite for blast transformation and more likely additional genetic events are required in the setting of disease progression.

Risk stratification in Myelofibrosis: Primary myelofibrosis (PMF) is the most aggressive among classical myeloproliferative neoplasm; its main features are bone marrow fibrosis, splenomegaly, and variable degrees of abnormal count of leukocytes, platelets and red cells (anemia). Current diagnosis is based on revised 2016 WHO criteria that allow to define two distinct diseases in terms of presentation and outcome: prefibrotic/early (pre-PMF) and overt fibrotic (overt PMF)(4). Despite the median survival of patients with PMF remains significantly worse than ET or PV, data derived from a large series of studies found a significant improvement over time in outcome during the last decade, increasing of almost 2 years (4.6 *vs* 6.5 yrs). Moreover, with respect to clinical, hematologic, and molecular phenotypes, pre-PMF is associated with better risk factors that result in prolonged overall survival compared to overt-PMF. All these features aside, prognosis is very heterogeneous with a median survival ranging from more than 15 yrs to less than 2 yrs. The most common causes of death include leukemic progression, that occurs in approximately 10-15% of cases, but many patients also die because of comorbilities including major cardiovascular events and consequences of cytopenias including infection or bleeding. Treatment is essentially clinical-oriented focusing mainly on the improvement of anemia, thrombocytopenia, constitutional symptoms and splenomegaly; the newly approved JAK1/2 inhibitor offered a new targeted therapy option for most of MF patients. Allogeneic hematopoietic stem cells transplantation (HSCT) is the only treatment that is potentially curative resulting in long-term remission, however mainly be-

cause of median older age only a minority of the patients are eligible. Taking the risk-benefit of this procedure into account, HSCT appears justified in otherwise patients whose expected median survival is less than 5 years; consequently considerable interest in accurate prognostication arise in the last few years aimed to identify patients with different life expectancy in order to select those that may benefit from HSCT versus other therapeutic (pharmacologic) alternatives. Therefore, primary myelofibrosis risk stratification is based on parameters predicting survival, and several efforts have been made to identify clinical and laboratory features that could predict patients' survival. The most widely used score is the International Prognostic Score System (IPSS), proposed in 2009 by the IWG-MRT(5); it is based on 5 variables: age >65 yrs, hemoglobin less than 10 g/dL, leucocyte count >25x10^9/L, peripheral blast cells count ≥1% and presence of constitutional symptoms. These prognostic factors formed the basis of four risk categories with non-overlapping survival curves: no factors (low risk), one factor (intermediate risk-1), two factors (intermediate risk-2) or three or more factors (high risk) where median survivals were 135 months, 95 months, 48 months and 27 months, respectively. IPSS is applicable at time of diagnosis and presented a higher discriminating power as well as accuracy and reproducibility than previous scoring systems, such as the Lille (or Dupriez) score proposed in 1996 or the prognostic model proposed in 2007 by Mayo Clinic in which two more prognostic factors were added: monocytosis and thrombocytopenia. However, as the IPSS estimates the survival at the time of diagnosis, a dynamic system able to reflect the variations occurring during disease course was warranted. At this regard, the dynamic IPSS (D-IPSS) uses the same prognostic variables of IPSS anytime the disease progression and is able to predict the remaining life expectancy even far from the diagnosis(6). The main difference in this score was to assign a doubled weight to anemia; risk categorization was accordingly modified in low (0 adverse points), intermediate-1 (1 or 2 points), intermediate-2 (3 or 4 points) and high (5 or 6 points). The corresponding median survivals were not reached, 14.2, 4.0 and 1.5 years for each category, respectively. Importantly, the DIPSS has also been shown to predict progression to acute leukemia and outcomes in patients receiving allogeneic HSCT. Dynamic IPSS was subsequently redefined by including three new parameters (the DIPSS-plus)(7): red cell transfusion need, platelet count less than 100x10^9/L, and "unfavorable" karyotype (included complex karyotype or sole or 2 abnormalities that included þ8, 7/7q-, i(17q), inv(3), -5/5q, 12p-, or 11q23 rearrangement) as additional independent risk factors. DIPSS-plus defined 4 risk categories with median survival of 15.4, 6.5, 2.9, and 1.3 years, respectively. Most recently, the phenotypic and prognostic relevance of "driver" and "non-driver" mutations was carefully investigated in a series of collaborative studies. In one of these studies involving 617 subjects with PMF a favorable effect of *CALR* mutations on survival and LFS was demonstrated; the study also documented the poorer survival and shorter LFS of triple-negative patients. Median survival was 17.7 years in *CALR*-mutant, 9.2 years in *JAK2*-mutant, 9.1 years in *MPL*-mutant, and 3.2 years in triple-negative patients. Furthermore, the impact of genetic lesions on survival was independent of the IPSS and DIPSS prognostic scoring systems. The prognostic advantage of *CALR* mutation in PMF regards only patients harboring type 1/type 1-like mutation, as the survival of those harboring type 2/type 2-like mutation does not differ from *JAK2*V617F-mutated patients. In recent years, a greater number of molecular abnormalities have been discovered to be associated with reduced survival in PMF. Mutation in at least one of *EZH2*, *ASXL1*, *IDH1/2* and *SRSF2* genes defined a "high molecular risk" (HMR) category associated with shorter survival(8). The study also found a prognostic relevance of the number of HMR adverse mutations; median survival was 2.6 years for patients with ≥2 mutations, 7 years for patients with 1 mutation, and 12.3 years for those with no mutations (LMR). The prognostic significance of HMR muted genes and the numbers of mutated genes were independent of both IPSS and DIPSS-plus and were also predictive of shorter LFS. Despite genetic information are helpful in predicting survival outcome or risk of disease complications, the prognostic value of these somatic mutations or abnormal karyotype have not been integrated yet with other clinical variables in multivariable analyses. Based on these seminal observations, two new prognostic models that incorporate clinical, hematological and mutational status data have been presented at the 2014 American Society of Hematology annual meeting. The Italian investigators of AGIMM group developed as proof of concept a "clinical molecular prognostic model" the "mutation-enhanced international prognostic scoring system (MIPSS)". The original study included 986 patients with PMF who were divided into learning (588 patients) and validation (398 patients) cohorts. An updated version of the MIPSS has been developed to be specifically directed to patients with prePMF and overtPMF younger than 70yr that are potential candidates to stem cell transplantation. The score included the following variables: leukocytes >25x10^9/L, platelets, <100x10^9/L, hemoglobin <10 g/dL, circulating blasts ≥2%, constitutional symptoms, BM fibrosis grade ≥2, absence of *CALR* type1/like and HMR category and number of mutated genes. Accordingly, 3 risk categories were delineated: Low, Intermediate and High (with a median survival of 27.7, 5.6 and 2.1 yrs, respectively. The second model was represented by an integrated genetics-based prognostic scoring system (GPSS)(9) that takes into account only age and karyotypic and mutational information. This model was used to delineate 4 risk categories: low, intermediate-1, intermediate-2 and high, with corresponding median overall survival of >17.0 years, 9.0 years, 5.0 years, and 2.2 years, respectively. Prognostic score developed for PMF are routinely used also in patients with post polycythemia vera (PPV)/essential thrombocytosis (PET) myelofibrosis. However, when these score were applied in PPV/PET-MF patients in retrospective studies, they failed to accurately discriminate different prognostic groups, suggesting a potential limitation of the IPSS/DIPSS in secondary MF. At this regard, it has also been postulated that the molecular landscape and the prognostic implication of mutations in PPV/PET-MF patients differ from primary MF. Concerning the prognostic impact of driver mutations, data from a large retrospective study in 359 patients (194 with PPV- and 165 with PET-MF) showed that driver mutations do not appear to meaningfully predict survival, at variance with PMF. In fact, survival among the mutational groups was largely superimposable, even between *CALR* type1/type1-like and type2/type2-like; the only exception was for triple negative patients that, as in PMF, presented the worst prognosis. In the above study mutations in HMR genes in secondary MF did not inform prognosis nor leukemia transformation, with the exception of *SRSF2* mutations that predicted for shorter survival in patients with PET-MF. Several studies, most limited in PPV-MF, reported a list of adverse prognostic factors for survival: unfavorable cytogenetics (abnormalities other than del13q or del20q), older age, hemoglobin <10g/dL, evolution from PV platelet count <100×10^9/L, leukocyte count >30×10^9/L and treatment with hydroxyurea at the time of diagnosis. The need of alternative tool required for secondary MF patients' risk stratification to help physicians in the therapeutic decision-making process led to the development of new prognostic score, the Myelofibrosis Secondary to PV and ET Prognostic Model (MYSEC)(10). The project collected retrospectively 685 (333 PET-MF, 352 PPV-MF) consecutive patients from 16 international centers in which multivariate analysis identified independent covariates as predictor of survival. Each variable was assigned a risk point: 0.15 points per each year of age, two points to hemoglobin <11g/dL, to circulating blasts ≥3%, and to *CALR*-unmutated genotype; one point to the presence of constitutional symptoms and to a platelet count <150x10^9/L. According to the sum of risk points ,patients were allocated in 4 risk categories: low, intermediate-1, intermediate-2 and high, with corresponding median survival times of not reached, 9.3 years, 4.4 years, and 2 years, respectively. A comprehensive list of the most well-validated prognostic scoring systems in PMF and secondary MF is reported in Table 2.

Conclusions: Prognostic factors and risk stratification systems in MPN have evolved significantly over the years, on one side thanks to carefully accomplished retrospective series of internationally collected patient cohorts, on the other following the discovery of driver and additional non-driver mutations and the advent of techniques like next generation sequencing (NGS). However, many challenges remain, in primis the lack of controlled prospective trials validating the impact of those scores in both the conventional and HSCT therapeutic setting. Second, mutation analysis is not widely available, and according to recent survey even driver mutations are not routinely searched in all patients. Finally, scores predicting survival in MF are useful only for selecting in advance patients potentially benefiting from HSCT, but do not inform currently alternative therapeutic options, and their value in modern times with JAK inhibitor(s) remain to be assessed. Under several respect, therefore, the apparent plethora of scores and the intense research activities behind reflect the lack of optimally performing prognostic variable.

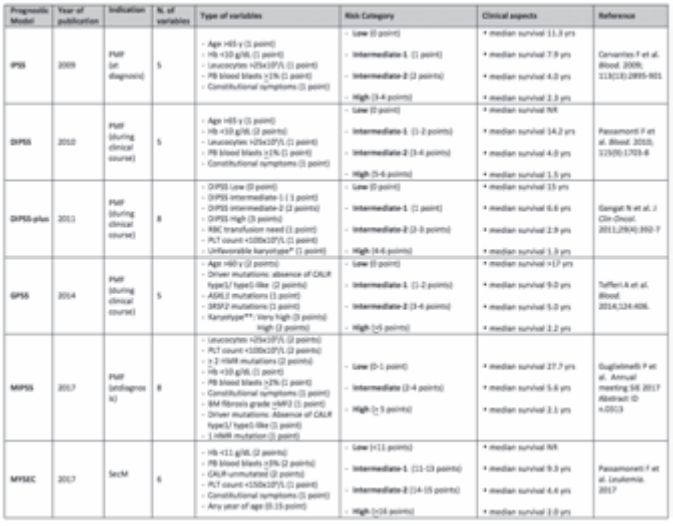
Table 1. Prognostic models in polycythemia vera and essential thrombocythemia.



Prognostic Model	Year of publication	Used for	N. of variables	Type of variables	Risk Category	Clinical aspects	Reference
Thrombotic Risk	2011	PV and ET	2	- Age >60 years - Previous thrombosis	- **Low** (no risk factor) - **High** (at least 1 risk factor)	• Incidence rate 2.03% (before 2005) and 2.24% (after 2005) pts/yrs • Incidence rate 4.01% (before 2005) and 2.93% (after 2005) pts/yrs	Barbui T et al. J Clin Oncol. 2011;29(6):761-770; Barbui et al. AJH. 2013; 90:934-7 Tefferi A. Blood 2006 Oct 1;108(7):2493-4 Alvarez-Larrán A et al. Blood 2010 116:1205-1210
Bleeding risk		ET	2	- PLT >1500x10^9/L - Previous major bleeding	- **High** (at least 1 risk factor)	• 0.04-0.05/100 pts-years in >60years pts • incidence rate ratio pts in under anti-platelet therapy: 5.4/1000 pts/yrs	
IPSET Thrombosis Score	2012	ET	4	- Age >60 years (1 point) - CV risk factors (1 point) - Previous thrombosis (2 points) - JAK2V617F (2 points)	- **Low** (0 - 1point) - **Intermediate** (2 points) - **High** (>3 points)	• probability of thrombotic events: 1.03% of patients/year • probability of thrombotic events: 2.35% of patients/year • probability of thrombotic events: 3.56% of patients/year	Barbui T et al. Blood. 2011;118(26):5128-5133.
Revised IPSET Thrombosis Score	2015	ET	3	- Age >60 years - Previous thrombosis - JAK2V617F	- **Very low**: (no risk factors) - **Low** (JAK2V617) - **Intermediate** (Age >60 years OR Previous thrombosis) - **High** (Age >60 years AND Previous thrombosis or Age >60 years OR Previous thrombosis AND JAK2V617F)	annual rate of thrombosis: • Very low w/o CV factors: 0.44%, • Very low with CV factors: 1.05% • Low w/o CV factors: 1.59% • Low with CV factors: 2.57% • High w/o JAK2V617F and CV factors: 1.44% • High with CV factors: 1.64% • High with JAK2V617F: 2.36% • High with JAK2V617F and CV factors: 4.17%	Barbui T et al. Blood Cancer J. 2015; 5: e369
Survival Risk	2013	PV	3	- Age : 57-66 (2 points); >67 (5 points) - Leukocytes >15x10^9/L (1 point) - Venous thrombosis (1 point)	- **Low** (0 point) - **Intermediate** (1-2 points) - **High** (>3 points)	• median survival 27.8 years • median survival 18.9 years • median survival 10.9 years	Tefferi A et al. Leukemia. 2013;27(9):1874-1881.
IPSET Survival	2012	ET	3	- Age >60 years (2 points) - Previous thrombosis (1 point) - Leukocytes >11x10^9/L (1 point)	- **Low** (0 point) - **Intermediate** (1-2 points) - **High** (3-4 points)	• median survival NR • median survival 24.5 years • median survival 13.8 years	Passamonti F et al. Blood. 2012;119(20):1197-1201.

PV= polycythemia vera; ET= essential Thrombocythemia; pts= patients; yrs=years; NR=not reached.

Table 2. Prognostic models in primary and secondary myelofibrosis.



Prognostic Model	Year of publication	Indication	N. of variables	Type of variables	Risk Category	Clinical aspects	Reference
IPSS	2009	PMF (at diagnosis)	5	- Age >65 y (1 point) - Hb <10 g/dL (1 point) - Leucocytes >25x10^9/L (1 point) - PB blood blasts >1% (1 point) - Constitutional symptoms (1 point)	- **Low** (0 point) - **Intermediate-1** (1 point) - **Intermediate-2** (2 points) - **High** (3-4 points)	• median survival 11.3 yrs • median survival 7.9 yrs • median survival 4.0 yrs • median survival 2.3 yrs	Cervantes F et al. Blood. 2009; 113(13):2895-901
DIPSS	2010	PMF (during clinical course)	5	- Age >65 y (1 point) - Hb <10 g/dL (2 points) - Leucocytes >25x10^9/L (1 point) - PB blood blasts >1% (1 point) - Constitutional symptoms (1 point)	- **Low** (0 point) - **Intermediate-1** (1-2 points) - **Intermediate-2** (3-4 points) - **High** (5-6 points)	• median survival NR • median survival 14.2 yrs • median survival 4.0 yrs • median survival 1.5 yrs	Passamonti F et al. Blood. 2010; 115(9):1703-8
DIPSS-plus	2011	PMF (during clinical course)	8	- DIPSS Low (0 point) - DIPSS intermediate-1 (1 point) - DIPSS intermediate-2 (2 points) - DIPSS High (3 points) - RBC transfusion need (1 point) - PLT count <100x10^9/L (1 point) - Unfavorable karyotype* (1 point)	- **Low** (0 point) - **Intermediate-1** (1 point) - **Intermediate-2** (2-3 points) - **High** (4-6 points)	• median survival 15 yrs • median survival 6.6 yrs • median survival 2.9 yrs • median survival 1.3 yrs	Gangat N et al. J Clin Oncol. 2011;29(4):392-7
GPSS	2014	PMF (during clinical course)	5	- Age >60 y (2 points) - Driver mutations: absence of CALR type1/ type1-like (2 points) - ASXL1 mutations (1 point) - SRSF2 mutations (1 point) - Karyotype**: Very high (3 points) High (2 points)	- **Low** (0 point) - **Intermediate-1** (1-2 points) - **Intermediate-2** (3-4 points) - **High** (>5 points)	• median survival >17 yrs • median survival 9.0 yrs • median survival 5.0 yrs • median survival 2.2 yrs	Tefferi A et al. Blood. 2014;124:406.
MIPSS	2017	PMF (endogenous)	8	- Leucocytes >25x10^9/L (2 points) - PLT count <100x10^9/L (2 points) - > 2 HMR mutations (2 points) - Hb <10 g/dL (1 point) - PB blood blasts >3% (1 point) - Constitutional symptoms (1 point) - BM fibrosis grade >MF2 (1 point) - Driver mutations: Absence of CALR type1/ type1-like (1 point) - 1 HMR mutation (1 point)	- **Low** (0-1 point) - **Intermediate** (2-4 points) - **High** (≥ 5 points)	• median survival 27.7 yrs • median survival 5.6 yrs • median survival 2.1 yrs	Guglielmelli P et al. Annual meeting SIE 2017 Abstract ID n.0013
MYSEC	2017	SecM	6	- Hb <11 g/dL (2 points) - PB blood blasts >3% (2 points) - CALR-unmutated (2 points) - PLT count <150x10^9/L (1 point) - Constitutional symptoms (1 point) - Any year of age (1-15 point)	- **Low** (<11 points) - **Intermediate-1** (11-13 points) - **Intermediate-2** (14-15 points) - **High** (≥16 points)	• median survival NR • median survival 9.3 yrs • median survival 4.4 yrs • median survival 2.0 yrs	Passamonti F et al. Leukemia. 2017

*PMF= primary myelofibrosis (both pre-MF and Overt-MF); SecM=secondary myelofibrosis (both post-polycythemia-MF and post-essential thrombocythemia MF); pts= patients; yrs=years; NR=not reached; RBC=red blood cell; *Unfavorable karyotype: 18,27/7q,i(17)q,25/5q,12p2,inv(3), 11q23 rearrangements). Hb= hemoglobin; PLT=platelet; PB=peripheral blood; TN= triple negative; HMR= High Molecular Risk; BM=bone marrow; ** Very high risk indicates monosomal karyotype, inv(3), i(17q), -7/7q-, 11q, or 12p abnormalities. High risk indicates complex non-monosomal karyotype, two abnormalities not included in the very high-risk category, 5q-,18, other autosomal trisomies except 19, and other sole abnormalities not included in other risk categories.*

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EXTRACELLULAR VESICLES

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Intercellular communication amongst neighbouring cells usually occurs either through cell-to-cell contact or exchange of soluble factors; the latter mechanism, differently from what one may think according to current experimental models *in vitro*, rarely occurs through the simple secretion of molecules in the intercellular microenvironment, which would lead to their rapid inactivation especially if released at tiny concentrations. A very effective, physiological intercellular communication (low molecule concentrations – maximum biological effect at both short and long range) is represented by the exchange of extracellular vesicles (EVs). EVs consist of a membrane-like envelope (spheric phospholipid bilayer) surrounding various molecules, such as proteins, DNA, different types of RNAs (mRNA and miRNA), lipids and active metabolites. EVs are shedding vesicles acting as molecular shuttles, constantly released by the cells in a sort of assembly chain process, which are characterized by different size and molecular content according to their origin and cellular functional status. EVs include exosomes (EXs, 50-100 nm), microvesicles (MVs, 100-1000 nm), apoptotic bodies (ABs, 50-500 nm) and some other membrane-bound particles (1-5). EXs originate from multivesicular body and contain common protein families such as chaperones (Hsp70 and Hsp90), cytoskeletal proteins (actin, myosin and tubulin), ESCRT proteins (TSG-101 and Alix), proteins involved in transport and fusion (Rab11, Rab7, Rab2 and Annexines) as well as tetraspanin proteins (CD9, CD63, CD81 and CD82) (1-5). MVs result from plasmatic membrane gemmation and contain specific cytoplasmatic proteins of the cells of origin, such as GTP-binding protein, ADP-ribosylation factor 6 (ARF6), matrix metalloproteinases (MMPs), glycoproteins (as GPIb, GPIIb-IIIa), integrins, receptors (e.g., EGFRvIII) and cytoskeletal components such as -actin and -actinin-4 (1-5). In addition, both EXs and MVs contain a large number of RNAs, whose functions are still under investigation (1-5). ABs are the result of the programmed cell death mechanisms and contain DNA, histones and cellular debris derived from cell dismantlement; AB formation is a highly controlled mechanism preventing leakage of potentially toxic, enzymatically active or immunogenic components of dying cells, thereby preventing tissue destruction, inflammation, and autoimmune reaction thrugh cytoskeleton weakening and activation of caspase enzymes. In addition, ABs act as a powerful signalling pathway for the microenvironment surrounding dying cells (1-5). A variety of cell types are capable of releasing EVs into the extracellular space both *in vivo* and

in vitro. Despite the increasing interest on EVs, their physiological role is still unclear, although it is widely accepted that they act as carrier of active biological molecules. To allow more accurate analysis, several diagnostic platforms have been developed, such as immunomagnetic exosome RNA (iMER) analysis, miniaturized micro–nuclear magnetic resonance (NMR) microfluidic chip system, Exochip, and label-free high-throughput nano-plasmonic exosome assay (nPLEX) using surface plasmon resonance (SPR). High-throughput procedures are under development for harvesting EVs from peripheral blood to turn EV research into routine diagnostics and therapeutic settings. Most of the studies regarding the possible patho-physiological roles of EVs have been based on indirect *in vitro* evidence, especially in the context of the immune system (1-5). EVs possess immunosuppressive effects on T cells and NK cells and play a crucial role in the induction of regulatory T and myeloid cells to further inhibit the immune response (2,4,6). EVs in co-culture with peripheral blood mononuclear cells (PBMCs) inhibit B cell proliferation and immunoglobulin release (2,4,6). Placenta-derived EXs, purified from the blood of pregnant women, carry immunosuppressive molecules, such as Fas-ligand, which induce tolerance towards the fetus (2,4,6). EVs may also stimulate the immune system, with the final effect depending on many factors, such as donor identity and target cells, as well as the biological context in which this interaction takes place (2,4,6). EV release occurs through both physiological and pathological processes. Numerous molecules shed through EVs are involved in the control of cell migration, proliferation, differentiation and apoptosis, hematopoietic stem cell development, coagulation, pregnancy or immune surveillance, as well as in carcinogenesis, tumor growth and metastasis (4,5,7-9). EVs shedding is a physiological phenomenon that includes cell activation and growth; furthermore, the presence of many stimuli, such as hypoxia, oxidative stress, and exposure to shear stress, can increase vesicle shedding (4,5,7-9). Different human diseases are mediated by EX release, including HIV-1 infection, autoimmunity, Parkinson's disease and Alzheimer's disease and many inflammatory reactions. In all these conditions EXs may represent both suitable biomarkers and potential therapeutic targets, once improved the specific identification and characterization of EXs amongst the other EV types. Tumor cells, such as pancreatic cancer cells (9), as well as leukemia cells release EXs during their development. Leukemia cell-derived EXs are involved in the bone marrow (BM) microenvironmental shift towards leukemia-supporting functions by promoting chemoresistance, preventing anti-leukemia immune response and eventually favouring leukemia relapse. Emerging evidence supports the role of EX in the development, progression and therapy of many other human hematological malignancies, such as myeloma and lymphoma (4,5,7-9). The effects of EVs were described in different inflammatory diseases. In 2014 a compassionate case of therapy-refractory GvHD was treated with EXs derived from mesenchymal stromal cells (MSCs) of four different BM-unrelated donors. The patient recovered within a few months after repeated treatments with allogenic MSC-derived EXs, suggesting that MSC-derived EXs may provide a potential cell-free and safe tool to treat GvHD and other inflammatory diseases (10). Nevertheless, so far, the main challenge still remains the precise characterization of the therapeutical EV content as well as the definition of the effective EV dose.

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ALLOGENEIC STEM CELL TRANSPLANTATION IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS: INDICATIONS AND EXPECTED RESULTS

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The indication to allogeneic stem cell transplantation (alloHSCT) has changed remarkably in the treatment strategy of Philadelphia positive (Ph+) chronic myeloid leukaemia (CML), while in Ph-chronic myeloproliferative neoplasms (MPNs) alloHSCT can be considered as an effective option in a selected proportion of patients with myelofibrosis (MF). Here we briefly review the most recent results achieved by alloHSCT in the treatment of these forms of chronic myeloproliferative disorders.

Allogeneic transplantation in CML: Before the introduction of tyrosine kinase inhibitors (TKIs), CML was the main indication for alloHSCT particularly for younger patients during the early chronic phase (CP) of the disease and possibly within the first year from diagnosis. In the last two decades the prognosis of this disease has dramatically changed thanks to the clinical approval in 2001 of imatinib, the first BCR-ABL TKIs (1). More recently, second generation TKIs (nilotinib and dasatinib) have been approved either for patients with CML intolerant or resistant to imatinib, as well as for newly diagnosis CML due to their higher efficacy in obtaining faster cytogenetic and molecular response in high risk patients (2, 3). Therefore, for the front line treatment of CP-CML patients, any of imatinib, nilotinib and dasatinib is recommended. Irrespectively of the TKIs used, the key point for the front line treatment is to achieve an "optimal" response because this endpoint is associated with a life expectancy close to that of the general population. Moreover, when a "failure" is documented, the patient should receive a different TKI based treatment with the aim to prevent transformation of disease that remains associated to a poor prognosis (4).

Table 1. Advice for consideration of alloHSCT for CML patients.

Disease Phase	Consideration for alloHSCT
Chronic phase CML	1. In patients with failure and/or intolerance to ≥2 prior TKIs 2. In patients with treatment failure on frontline nilotinib or dasatinib 3. In patients with aT315I mutation
Accelerated-phase CML	1. In all patients who do not have an optimal response to TKIs 2. In all patients who progress to accelerated phase during TKIs therapy
Blastic-phase CML	1. In all patients following TKIs therapy alone or in combination with chemotherapy

By this approach, the probability to achieve an excellent hematologic or even molecular control of the disease is so good that, no one patient should be immediately put at risk of a transplant related death or graft *versus* host disease (GvHD). Nonetheless, despite a such outstanding progress of the medical treatment, a distinct group of CML patients still require allogeneic transplant that remains a definitive treatment option for CML. Indeed, according to National Comprehensive Cancer Network (NCCN) and the European LeukemiaNet (ELN) alloHSCT remains the option for accelerated-phase (AP) or blastic-phase (BP) and selected CP-CML patients, such as those with poor responses to TKIs, unable to tolerate TKIs or with a T315I mutation (4, 5). For patients presenting with or evolving into AP or BP, the clinical outcome with medical therapy is significantly poorer. Such patients should receive preferably second generation TKIs (nilotinib or dasatinib) with or without the addition of chemotherapy for BP patients to achieve at least a good hematologic response and transplant should be always considered as an early treatment opportunity. Of remarkable interest is the notion that the clinical outcome of alloHSCT in CP-CML has progressively improved during the past few years. The Gruppo Italiano Trapianto di Midollo Osseo (GITMO) data showed that first CP-CML patients receiving alloHSCT between year 2005-2012 (all being previously treated with TKIs) had a 75% 2 year overall survival, no matter if the stem cell source was from a matched sibling or unrelated donor. On contrast, the overall rates of cure for AP- and BP-CML remains below 40% and 20%, respectively, but for these high risk patients alloHSCT remains

the only curative treatment. Table 1 summarized the generally accepted current indications for alloHSCT in CML.

Allogeneic transplantation in Myelofibrosis: Myelofibrosis is the most aggressive form among Philadelphia chromosome-negative (Ph-) MPNs characterized by anemia, extramedullary hematopoiesis, constitutional symptoms, bone marrow failure and an increased risk of transformation into acute myeloid leukaemia (AML). When the disease enters this late stage, the expected survival is short and alloHSCT represents currently the only curative treatment. During the past years the transplant activity for MF has been growing significantly despite the persistent concern about the non-relapse mortality that remains significantly high in most studies and requires a careful risk evaluation at the individual patient level. Therapy of MF is conventionally based on the patient risk category and several prognostic scoring systems based on clinical/hematologic and, more recently, cytogenetic parameters have been validated, such as IPSS, DIPSS and DIPSS plus, which defines risk groups with different probabilities of survival. The recent consensus guidelines by European LeukemiaNet and European Blood and Marrow Transplantation Group recommend that patients with intermediate-2 or high-risk disease, in which median survival is usually less than 5 years, and age <70 years should be considered as candidates for alloHSCT. Patients with intermediate-1-risk disease and age <65 years should be considered as candidates if they present with either refractory, transfusion-dependent anemia, or a percentage of blasts in peripheral blood >2%, or adverse cytogenetics, as defined by the DIPSS-plus classification (6). In MF, the driver mutational profile has prognostic implications and, recently, the mutational status appears to be more complex, due to the emergence of new data regarding the presence of additional somatic mutations, such as *TET2*, *ASXL-1*, *IDH, SRSF2* or *EZH2*, which can precede or follow the acquisition of MPNs driver mutations (7, 8). The possibility to integrate clinical information derived from the new molecular mutations into prognostic models may improve the prognostic stratification, as a basis for a correct treatment planning. In this regard, by combining *CALR*, *JAK2*V617F, *MPL*W515L/K, triple-negative status, each single variable included in the IPSS, and additional mutations (*ASXL-1*, *SRSF2*, *EZH2*, and *IDH1/2*) a new mutation-enhanced IPSS (MIPSS) has been reported. This new score allows to distinguish 4 different groups without overlap in their survival according to the variables (9) (Table 2). A second new prognostic model GPSS (genetics-based prognostic system) that takes into account only age, karyotyping and mutational information has been developed (Table 2).

Table 2. New molecular based risk stratification in Myelofibrosis.

Variable	MIPPS[9]	GIPPS[10]
Age>60	✓ (1.5 points)	✓ (2 points)
Constitutional Symptoms	✓ (0.5 points)	No
Hemoglobin < 10g/dl	✓ (0.5 points)	No
Platelets < 200 x 10^9/L	✓ (1.0 points)	No
Triple negative	✓ 1.5 (points)	✓ (2 points)
JAK2 or *MPL* Mutation	✓ 0.5 (points)	✓ (2 points)
ASXL1 Mutation	✓ 0.5 (points)	✓ (1 points)
SRSF2 Mutation	✓ 0.5 (points)	✓ (1 points)
CALR Type 2, Type 2 like	-	✓ (2 points)
Unfavorable Cytogenetics*	-	✓ (3 points for very high risk; 2 points for high risk)
Scoring risk factor	Low = 0 to 0.5, Intermediate-1 = 1 to 1.5, intermediate-2 = 2 to 3.5, high = ≥4	Low = 0, intermediate-1 = 1 to 2, intermediate-2 = 3 to 4, high ≥5

**Very high risk: monosomal karyotype, inv(3), i(17q), -7/7q-, 11q, or 12p abnormalities; high risk: high risk indicates complex non-monosomal karyotype, 2 abnormalities not included in the very high-risk category, 5q-,+8, other autosomal trisomies except +9, and other sole abnormalities not included in other risk categories. Intermediate: sole abnormalities of 20q-, 1q+, or any other sole translocation, and –Y or other sex chromosome abnormality; low risk: normal cytogenetics or sole abnormalities of 13q- or +9.*

This model delineates four risk categories associated with different survival, ranging from more than 17 years to 2.2 years (10). These first attempts to integrate mutation information into the available prognostic scoring system remark the practical value of mutational profiling to refine prognostic stratification of patient with MF for a correct treatment planning, including the role of alloHSCT in patients traditionally categorized as low- or intermediate-1. In addition, the presence of patient specific molecular markers might offer the possibility to monitor the depth of remission by a careful molecular evaluation of minimal residual disease (MRD). This opportunity allows to start an early preemptive therapy as soon as any evidence of disease reappraisal is documented. At present, even if outcome of transplantation in MF is improving due to the introduction of less toxic conditioning regimens and optimization of remission monitoring techniques and relapse prevention strategies, the decision regarding alloHSCT in the category of patients with low- or intermediate-1 risk disease should be individualized taking into consideration also patients' preferences (e.g. more concern about major complications of alloHSCT, such as rejection, GvHD and infections, rather than the risk of leukemic transformation or decreased survival).

Conclusions: The success of TKIs therapy significantly reduced the indication for an alloHSCT for patients with CP-CML. However, patients who do not tolerate TKIs, progress, despite second- or third-line TKIs and develop TKI resistant mutations still require transplant that remains a curable treatment options for an otherwise incurable disease. Most recent results suggest that the outcome after transplantation is improving particularly for patients who remain in a chronic phase of their disease despite a modest or poor response to TKIs. Considering that alloHSCT is currently the only treatment for which a definitive curative potential has been demonstrated in MF, the ability to identify patients with high-risk disease is crucial for transplantation clinical decision. Patients with intermediate-2 or high risk disease according to DIPSS and age <70 years should be considered as candidates for alloHSCT. With the recent coming of new molecular techniques, the mutational landscape of MF continues to evolve with the identification of additional recurrent mutations. The prognostic relevance of these mutations is currently in progress and might be useful to identify patients with high-risk disease as a tool for a correct treatment planning based on individualized patient features.

RECOMMENDATIONS FOR THE CLINICAL PRACTICE: THE SIE PROJECT

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Ematologia, SOC Oncologia, Ospedale C Massaia, Asti

Best available scientific evidence is the basis for practice recommendations provided by clinical practice guidelines. As evidence is rapidly increasing, the number of guidelines supported by Hematology scientific societies is also growing up. Nevertheless, recommendations provided by different guidelines are often ambiguous and inconsistent. Furthermore, applicability of recommendations is hampered by content-related issues, eg. country-specific regulatory limitations, timely updating, and by access-related issues, eg. Pdf format. Finally, textual recommendations can be hardly tailored to the specific patient (see GuideLine Implementability Appraisal). The consequence of the above limitations is a low adherence to clinical practice recommendations. The Italian Society of Hematology launched the first guideline project in the year 2002 adopting the SIGN evidence paradigm, which was replaced by the GRADE paradigm in the year 2009: all the guidelines were fully published in a static pdf format and updated by subsequent full paper documents. In the year 2014 the SIE guideline effort moved to harmonization of existing national and international guidelines, according to the ADAPTE methodology. Guidelines were retrieved through a systematic search and checked for quality, updating and completeness: selected guidelines provided the basis for the elaboration of PICO-based recommendations. A limited set of recommendations was agreed upon by the working group and fully validated by an Advisory Panel including SIE members and physicians belonging to other specialties (for multidisciplinary issues). The SIE project also aimed at pro-

ducing computerized evidence-based recommendations that guaranteed a readily accessible and timely updatable reference besides a full support to unambiguous tailoring of recommendations to patients' clinical features. Therefore, the SIE web site was planned to host interactive flow charts featuring in a step-wise sequence the decision criteria and the appropriate clinical options in each patient subgroup. Flowcharts were equipped with 50 dynamic online calculators of prognostic and frailty scores in order to purse the full adherence to specific decision criteria. Evidence supporting each recommendation was both integrated into the web pages and fully exposed in an attached pdf format report. Seven evidence-based charts were completed and published online: 1) first-line therapy for elderly patients with chronic lymphocytic leukemia; 2) ruxolitinib therapy for myelofibrosis patients; 3) diagnosis and therapy of iron deficiency anemia; 4) screening, monitoring and prophylaxis of infections; 5) first-line therapy for mantle-cell lymphoma; 6) first-line therapy for acute myeloid leukemia; 7) prevention of tumor lysis syndrome. Updates to the recommendations are checked tice in a year and can be prompted by new regulatory issues. Access to the recommendations will be monitored and single audits will be implemented in order to verify the preferences of SIE members. Fully dynamic online evidence-based recommendations can support hematologists' clinical practice. Harmonization of international guidelines and fully algorithmic representation of knowledge by interactive charts may foster rapid and integrated decision support for non-expert hematologists. The SIE project plans to expand fully in order to provide a full platform for Italian hematology practice.

MANAGEMENT OF BLEEDING ASSOCIATED WITH VITAMIN-K ANTAGONISTS (VKA) AND DIRECT ORAL ANTICOAGULANTS (DOAC)

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Every anticoagulant treatment, although effective in reducing thromboembolic risk, carries a non-negligible risk of clinically relevant bleeding, first of all intracranial haemorrhage (ICH). Regarding vitamin-k antagonists (VKA), the risk of major bleeding ranges between 1 and 10 cases for 100 patients every year, depending on the clinical characteristics of the group studied as well on the method of data collection [1,2]. The incidence of AVK-related ICH ranges from 0.25 to 1.1%/year [1], but can reach 2% when the INR exceeds 2.0, and rises dramatically thereafter [3]. Recently published trials evaluating Direct Oral AntiCoagulants (DOAC) for the prevention of peripheral embolism in patients with atrial fibrillation (AF) have shown that these drugs significantly reduce all-cause mortality (0·90, 0·85–0·95; p=0·0003) and intracranial haemorrhage (0·48, 0·39–0·59; p<0·0001), but increase gastrointestinal bleeding (1·25, 1·01–1·55; p=0·04) as compared with AVK [4]. Despite their good safety profile and their short half-life, that allows a rapid clearance from the circulation, bleeding remains a major concern also with DOAC, first of all because of the lack of sound evidence about the optimal management of haemorrhage associated with their use. The aim of this paper is to provide some basic concepts as well as practical suggestions about the management of VKA and DOAC-associated bleeding. *Classification of bleeding:* A careful and uniform quantification of antithrombotic treatments associated bleeding can be challenging, but it is also a necessary requirement in order to provide the patients with the most appropriate therapy, balancing haemorrhagic and thrombotic risk. Indeed, in 2005 the Subcommittee on control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis [5] recommended the following criteria for the definition of major *bleeding* in non-surgical patients receiving antithrombotic agents: Fatal bleeding, and/or Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intra- articular or pericardial, or intramuscular with compartment syndrome, and/or Bleeding causing a fall in haemoglobin level of 20 g/L or more, or leading to transfusion of two or more units of whole blood or red cells [5]. Such a classification represented a relevant improvement toward the standardization of bleeding episodes, but it is unable to provide a reliable tool in order to assess the best treatment for patients experiencing a haemorrhagic complication of antithrombotic treatments. Indeed, a fall in haemoglobin level of 20 g/L or more is certainly relevant in order to assess the safety of a new anticoagulant drug, but it requires a different clinical approach, first regarding the emergency reversal of the effect of the drug, depending on the haemoglobin starting level as well as on the patient's thromboembolic risk. Relevant to this, the BCSH restricted the definition of major bleeding in terms of anticoagulation reversal only to the limb or life-threatening bleeding that requires complete reversal within 6–8 h [6]. On this quite puzzling scenario, in order to simplify the practical approach to the problem in terms of management of antithrombotic drugs, we suggest to classify anticoagulant-related bleeding events on three categories: minor bleeding, non-life or organ-threatening major bleeding, and life- or organ threatening-major bleeding. *Minor bleeding:* Is any bleeding that, although symptomatic, does not fulfil the criteria for being classified as "major, *i.e.* haematochezia, epistaxis, ecchymosis, conjunctival haemorrhage, haematuria not determining anaemia. Usually minor bleeding does not require specific treatments, except the reduction of VKA dose if INR is over the therapeutic range, and a more strict clinical control. Patients bleeding on therapeutic levels of anticoagulation should be investigated for the source of bleeding, namely in case of haematuria, which is not a feature of anticoagulation. For bleeding into the oral cavity, antifibrinolytic drugs, such as tranexamic acid mouthwash, are often very helpful. For epistaxis, nasal packing can be useful when simple measures fail. According to the ISTH classification, major *bleeding* is any symptomatic bleeding in a critical site and/or determining a fall in haemoglobin level of 20 g/L or more, or leading to transfusion of two or more units of whole blood or red cells [5]. Often, but not always, major bleeding can be life or organ threatening, and the management of such bleeding in terms of reversal of anticoagulant treatment should be tailored according to the severity of haemorrhage. If the haemorrhage can be classified as major but it is neither life- nor organ-threatening, a more conservative approach can be adopted, consisting of general supportive measures, not requiring the immediate reversal of the anticoagulant activity (Table I).

Table 1. Suggested management of major bleeding, non life or organ threatening, occurring during anticoagulant therapy with vka or doac.

- Withhold anticoagulant treatment
- Evaluate concomitant medications
- Provide mechanical or surgical hemostasis if required, according to the local resources
- Provide supportive measures:
  - Fluid resuscitation and transfusion of blood products if needed
  - Maintain diuresis
- Assess the anticoagulant activity of the drug by specific tests (see text)

- **Patients on VKA:**
  - Give 10 mg intravenous vitamin K

- **Patients on DOAC:**
  - Assess the type and the dose of DOAC
  - Assess the time of the last assumption of the drug
  - Check Glomerular Filtration Rate (GFR) by Cockroft-Gault's equation:
    - If GFR >50 ml/min, given the short half-life of DOAC, a normal haemostasis is expected to occur in 24 hours, and only supportive measures can be adopted
    - If GFR <50 ml/min, the half-lives of DOACs are prolonged in a drug-dependent extent, and the use of an antidote can be considered if the patient has ongoing bleeding (see Table III).

The need for the assessment of the actual anticoagulant activity worths some more discussion. There is no doubt that this is recommended for the management of bleeding in VKA-treated patients, given the wide availability of INR also in an emergency setting.On the other hand, much more uncertainty exists about the need for the same assessment also for DOAC. The limited availability of specific assays calibrated for each of the DOACs and their long turnaround time may render them of little value during acute bleeding. Moreover, the wide inter- and intra-individual variability of plasma levels of DOAC [7] and the lack of sound evidence about the therapeutic ranges of these drugs limit the possibility of using the results of such assays for the manage-

ment of actively bleeding patients. This is certainly true in the case of life-threatening bleeding, such as ICH, or in patients requiring emergency surgery. Except for these patients, the results of laboratory test specifically measuring the anticoagulant effects of the DOAC or the plasma drug concentrations, now available also in an emergency setting, is highly advisable in order to decide whether an antidote is indicated or not. Certainly such a clinical decision must be guided first by the time since the last intake of the DOAC and by the determination of the glomerular filtration rate, which influences the half- lives of the DOACs. However, in the recently published RE-VERSE AD trial evaluating the use of idarucizumab for reversal in patients acutely bleeding while on treatment with dabigatran, 113/494 patients had a normal dilute thrombin time at study enrollment, suggesting very low or absent concentration of the drug at that moment. This highlights the need for the availability of specific assays for assessing DOAC activity in every emergency department, in order to identify patients who are not likely to benefit from high cost antidotes.[8] *Suggested management of life- or organ threatening- major bleeding occurring during anticoagulant therapy with vka:* The issue of the management of VKA-related major bleeding has been already addressed by several guidelines [6, 9], whose recommendations are summarized in Table II. Relevant to this issue, it should be highlighted that Prothrombin Complex Concentrate (PCC) allows a faster and more complete correction of the AVK-related coagulopathy than fresh frozen plasma (FFP), and is therefore the first choice in case of life-threatening bleeding. Indeed, it has been recently demonstrated that the use of PCC for warfarin reversal, as compared to FFP, is associated with a significant reduction in all-cause mortality, more rapid INR reduction, and less volume overload without an increased risk of thromboembolic events [10].

Table 2. Suggested management of major bleeding, life or organ threatening, occurring during anticoagulant therapy with vka.

- Provide general supportive measures indicated in Table I
- Give 10 mg intravenous vitamin K.
- Give intravenous prothrombin complex concentrate (PCC). The dose of PCC should be determined as follows:

If INR not available
- PCC: 20 IU/Kg

If INR available the dose of PCC is determined as follows:
- INR 1.5 - 2.0: PCC 20 IU/Kg
- INR 2.1 - 3.9: PCC 30 IU/Kg
- INR 4.0 - 5.9: PCC 40 IU/Kg
- INR >6: PCC 50 IU/Kg

- Avoid PCC if INR<1.5
- Fresh Frozen Plasma produces suboptimal anticoagulation reversal and should only be used if PCC is not available

Specific antidotes for direct oral anticoagulants associated bleeding: Conventional anticoagulants VKA have numerous limitations, but on the other hand their effect can be rapidly and completely reversed by either vitamin K and coagulation factor concentrates. This does not occur with DOAC, and the lack of tested reversal agents, and the consequent fear of uncontrolled bleeding, have represented a matter of concern in the clinical setting. The short half-life of DOAC in healthy patients should allow, in non-urgent situations, a relatively rapid reversal simply through the discontinuation of treatment. Nevertheless, in many emergency situations the simple discontinuation of a DOAC is insufficient to address the clinical need, such as in case of life- threatening bleeding, urgent interventions and trauma, particularly in elderly patients with some degree of renal impairment. Three antidotes for the DOACs are under various stages of development. Idarucizumab (Praxbind), the antidote for dabigatran, is now licensed in the United States and Europe [8, 11]. Andexanet alfa, the antidote for the oral factor Xa (FXa) inhibitors, is undergoing phase III investigation [12]. Ciraparantag (PER977), an agent reported to reverse the anticoagulant effects of all of the DOACs, is at an earlier stage of development [13]. *Idarucizumab:* Idarucizumab is a humanized monoclonal antibody fragment that neutralizes dabigatran activity by binding it with 350-fold higher affinity than that of this drug for thrombin. Idarucizumab and idarucizumab-dabigatran complexes are cleared by the kidneys, as is dabigatran. After intravenous infusion, the half-life of idarucizumab is about 45 min in subjects with normal renal function [8,11]. Idarucizumab was recently approved in the United States, Canada, and European Union under an accelerated approval based on preliminary results of the Reversal Effects of Idarucizumab on Active Dabigatran (RE-VERSE AD) study. RE-VERSE AD was a multicentre, prospective, single-cohort study assessing the efficacy and the safety of idarucizumab in patients experiencing uncontrollable or life-threatening bleeding (group A) or requiring surgery or other invasive procedures that could not be delayed for at least 8 hours and for which normal haemostasis was required (group B) [14]. All patients received 5 g of intravenous idarucizumab, which was administered as two 50-ml bolus infusions, each containing 2.5 g of idarucizumab, no more than 15 minutes apart. The primary efficacy end point was the maximum percentage reversal of the anticoagulant effect of dabigatran. Clinical outcomes, as assessed by the treating clinician, were secondary end points: the extent of bleeding and hemodynamic stability for the group A and the degree of periprocedural hemostasis for the group B. Very recently, data about the entire cohort of 503 patients included in that study have been published [8], confirming the previously published results of the interim analysis [15]. Idarucizumab was able to rapidly and completely reverse anticoagulation in more than 98% of the patients. The time to the cessation of bleeding could be assessed in only 44% of patients in group A; among them, the median time to haemostasis after the administration of idarucizumab was 2.5 hours (95% CI, 2.2 to 3.9). Among the 197 patients in group B who underwent surgery or an intervention, periprocedural hemostasis was assessed as normal in 184 patients (93.4%), mildly abnormal in 10 (5.1%), and moderately abnormal in 3 (1.5%); no patients had severely abnormal haemostasis. There were no reports of hypersensitivity. At 90 days follow-up, 6.3% of patients (31/494) had experienced a thrombotic event, including 8 ischaemic strokes, 7 myocardial infarctions, 15 venous thromboembolisms, and 1 systemic arterial embolism. Overall, these results show the possibility of rapidly and safely reverse the anticoagulant effect of Dabigatran in an emergency situation. However, some relevant issues need further research, as REVERSE-AD trial has two major limitations, *i.e.* the lack of a control group and of rigorous criteria for assessing the attainment of effective haemostasis. Moreover, it has been recently showed that repeated doses of idarucizumab may be necessary in cases of massive dabigatran accumulation, mainly because of renal failure [16]. In such rare, but compelling cases, the monitoring of dabigatran level after Idarucizumab infusion is crucial to detect rebound and to consider the combination of antidote application and renal replacement therapy. *Andexanet:* Andexanet alfa is a recombinant human FXa variant with the active-site serine residue replaced with alanine to eliminate catalytic activity and with the membrane-binding domain deleted to prevent incorporation into the prothrombinase complex [12]. Andexanet serves as a decoy for the oral FXa inhibitors because it binds them with affinities similar to those of native FXa.

Table 3. Suggested management of major bleeding, life or organ threatening, occurring during anticoagulant therapy with vka.

For patients on Dabigatran treatment:
- Administer two 50-ml bolus infusions, each containing 2.5 g of idarucizumab (Praxbind®) intravenously, no more than 15 minutes apart.

For patients on F-Xa inhibitor treatment:
- Administer prothrombin complex concentrate (PCC) at 25 IU/kg doses, eventually repeatable once-twice after accurate evaluation of thrombotic risk;
- Administer tranexamic acid at 15 mg/kg doses 3 times daily intravenously or 25 mg/kg three times a day until bleeding control;
- In case of bleeding non responsive of previous treatment, consider the possibility of giving active prothrombin complex concentrate (FEIBA®) at indicative doses of 50 IU/kg until a maximum of 200 IU/kg daily;

These measures can be taken in case of life- or organ-threatening major bleeding in presence of abnormal specific laboratory test results (diluted thrombin time or anti-IIa chromogenic assay). In cases with reliable anamnestic information regarding DOAC intake, if specific assay results can't be available in time according to clinical patient's conditions, the above mentioned measures should be adopted immediately.

The safety and efficacy of Andexanet-alfa for reversal of F-Xa inhibitor (apixaban, rivaroxaban, edoxaban, or enoxaparin) are undergoing evaluation in the ANNEXA-4 trial [17]. In this ongoing, multicentre, prospective, open-label, single-group study supported by Portola Pharmaceuticals, adult patients with acute major bleeding within 18 hours of administration of a FXa inhibitor (apixaban, rivaroxaban, edoxaban, or enoxaparin) received an andexanet bolus during a period of 15 to 30 minutes, followed by a 2-hour infusion of the drug. The two co-primary outcomes were the percent change in the anti–factor Xa activity and the rate of excellent or good haemostatic efficacy 12 hours after the andexanet infusion, as defined on predetermined criteria adapted from those used in a previous study evaluating prothrombin complex concentrate [18]. The results of an interim analysis of 67 patients have shown a good treatment efficacy, with a relevant (about 90%) decrease in median anti–factor Xa activity both after bolus and during the 2-hour infusion. However, four hours after the end of the infusion, there was a relative decrease from baseline of 39% in the measure of anti–factor Xa activity among patients receiving rivaroxaban and of 30% among those receiving apixaban [17]. The rate of excellent or good haemostatic efficacy 12 hours after the andexanet infusion was 79%. Thrombotic events occurred in 12 of 67 patients (18%) during the 30-day follow-up. Despite these encouraging results, some concerns arise from the rate of thrombotic events during the follow-up, and therefore a controlled study would be advisable to assess whether the frequency of these events exceed that expected in patients already at increased risk for thromboembolism. *Ciraparantag:* Ciraparantag is a small, synthetic, water-soluble, positively charged molecule able to bind dabigatran, rivaroxaban, apixaban, and edoxaban via hydrogen bonds [13, 19]. In a phase 1 study in healthy volunteers a single 60-mg oral dose of edoxaban, an intravenous bolus of ciraparantag dose-dependently shortened the whole blood clotting time to within 10% of baseline and restored normal clot architecture based on scanning electron microscopic analysis [20]. So far, no clinical data about the efficacy and safety of ciraparantag on actively bleeding patients are available.

Suggested management of life- or organ threatening- major bleeding occurring during anticoagulant therapy with doac: As for VKA, the issue of how to treat a patient experiencing a life-threatening bleeding during anticoagulant treatment by DOAC has been addressed by several guidelines [21-23], whose recommendations are summarized in Table III. However, differently from VKA, good clinical evidence from studies in actively bleeding patients are restricted to the use of idarucizumab for reversing dabigatran effect. In the absence of a specific agent for the specific reversal of factor Xa ihibitors, a number of strategies have been proposed, including the use of PCC or activated PCC (APCC). However, it should be observed that none of these agents has been prospectively studied in bleeding patients, and current evidence for their efficacy is based on a range of animal models and studies in healthy volunteers (24). Well designed, high quality studies are urgently needed in order to improve our knowledge about many issues crucial for patients management, such as the efficacy and safety of PCC for DOAC-associated bleeding, and the cut-off level (if available) to properly administer the antidote or to safely perform an emergency invasive procedure.

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NOVEL THERAPEUTIC TARGETS IN ACUTE MYELOID LEUKEMIA

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Introduction: Acute Myeloid Leukemia (AML) is a hematologic malignant disorder with clonal proliferation of myeloid precursors incapable of differentiating into more mature, functioning cellular elements, and impaired normal hematopoiesis(1). AML mostly affects elderly adults with a median age at diagnosis of 68 years and one third of patients older than 75 years, which explains why the disease prevalence is constantly increasing, in accordance with the prolongation of life expectation, with predicted new 21,380 cases in Europe in 2017(1). Intensive induction chemotherapy followed by either consolidation chemotherapy or allogeneic hematopoietic stem cell transplantation (allo-HSCT), once the complete remission (CR) is achieved, remains the standard treatment for AML(1). Patients considered unfit because of their age and /or performance status may be given either less intense therapies with palliative intent (e.g. hypomethylating agents) or best supportive care. Prognosis is generally poor. In patients younger than 60 years, the CR rates are 60–80%, but the overall cure rates are only 35-40%(1).

Elderly patients and those with adverse karyotypes have even lower CR rates (35-50%) and the cure rates are less than 10%(1). Thanks to recent, impressive progresses in DNA sequencing techniques that have led to a better understanding of the key genetic drivers of AML, it is now clear that AML is a heterogeneous disease consisting of many genetically unique subtypes, each with a distinct clinical outcome(2-4). Improved genetic testing has already revolutionized risk stratification and is of vital importance for identifying which patients would best benefit from allo-HSCT (Table 1)(1). Besides the progresses in the molecular characterization of the disease, AML general therapeutic strategy has remained essentially the same in the last 40 years. With the significant exception of acute promyelocytic leukemia treatment which now consists of all-trans-retinoic acid and arsenic trioxide, approved in 1995 and 2001 respectively, there was no new US Food and Drug Administration (FDA) drug approval for AML until last year since idarubicin in 1990. In 2000 gemtuzumab ozogamicin had actually been approved but it was withdrawn from the market 10 years later. The knowledge of the full AML genome landscape and our improved understanding of the underlying biology of AML will help to identify new oncogenic pathways that may serve as new therapeutic targets, opening the possibility for developing targeted and more effective treatments. Just very recently new therapeutic approaches and novel therapeutic strategies have been developed and have demonstrated promising results. At present, however, there are only few examples of molecularly targeted therapies for AML. The most paradigmatic one is based on the differentiating agent all-*trans*-retinoic acid (ATRA) that, when combined with chemotherapy or with arsenic trioxide (ATO), has dramatically changed the outcome of patients affected by acute promyelocytic leukemia (APL), allowing cure rates above 90%(5). The success obtained in the specific genetic subtype APL indicates that a molecularly targeted therapy is possible in AML, and can lead to high-rate cure of patients. As exciting and hopeful this may sound, it is important to highlight that, as for now, traditional chemotherapy, because of its independence from the mutational complexity of each patient compared to precision medicine drugs, remains the gold standard of AML treatment.

Table 1. 2017 ELN risk stratification by genetics(1).

Risk category	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low} Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high} Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low} (without adverse-risk genetic lesions) t(9;11)(p21.3;q23.3); *MLLT3-KMT2A* Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214* t(v;11q23.3); *KMT2A* rearranged t(9;22)(q34.1;q11.2); *BCR-ABL1* inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)* -5 or del(5q); 27; -17/abn(17p) Complex karyotype, monosomal karyotype Wild-type *NPM1* and *FLT3*-ITD^{high} Mutated *RUNX1* Mutated *ASXL1* Mutated *TP53*

Genomic landscape of AML: The Cancer Genome Atlas profiled 200 cases of *de novo* AML by whole-genome (n=50) or whole-exome (n=150) sequencing, along with RNA and microRNA sequencing and DNA-methylation analysis(2). This study lead to the discovery of 23 driver recurrent mutations, several of which (e.g. *NPM1* and *FLT3*-ITD mutations) had been previously identified through different approaches. AML mutations appear to be organized into 9 functional categories including transcription-factor fusions, tumor suppressors, DNA-methylation–related genes, activated signaling genes, chromatin-modifiers, myeloid transcription-factors, cohesin-complex genes, spliceosome-complex genes and *NPM1* which defines its own category(2). As previously reported, the most frequently mutated genes in cytogenetically normal AML (CN-AML) are *nucleophosmin* (*NPM1*) (about 60% of cases)(6), *FMS-like tyrosine kinase 3* (*FLT3)* and *DNA N-methyltransferase 3A (DNMT3A)* (each in approximately 30% of cases). Other genes (e.g. *Isocitrate dehydrogenase 1/2, IDH1/2*; *neuroblastoma-RAS, NRAS; tet-methylcytosine-dioxygenase 2, TET2*) are mutated at lower frequency. Among the most frequent CN-AML associated mutations, only those affecting the *NPM1* or double *CCAAT/enhancer-binding protein alpha (CEBPA)* genes are associated with distinct features and recognized as distinct entities in the revised WHO classification of lymphohemopoietic neoplasms(7). The use of genetic profile for disease classification and in the clinical practice is an active field of research(4). On one side, genetic markers may represent important prognostic factors and may serve to detect minimal residual disease (MRD); on the other side, although a genetic marker may not have prognostic relevance, its presence may provide a target for novel therapies.

Basic concepts of "therapeutic target": The main goal of a targeted therapy is to have more efficient drugs to cure a specific disease, but also to find out drugs that more specifically kill leukemic cells possibly sparing normal cells, with subsequent reduction in drug-related toxicities. Most of the chemotherapeutic agents used today to treat cancer have remarkably low therapeutic indices and narrow therapeutic windows. To develop drugs with a high therapeutic index for cancer treatment is a major challenge. Many factors influence the therapeutic index of a drug. Some relate to the quality of the drug itself - for example, 'off-target' effects due to hit of unintended targets. Others relate to the nature of its target. To have a high therapeutic index and selectively kill cancer cells an anticancer drug should hit targets that are essential for the viability of cancer cells but are not present in normal cells (the so-called 'target-driven therapeutic index')(8). In that sense, the fusion proteins generated by cancer-associated chromosomal translocations or mutated oncoproteins due to cancer-associated gene mutations seem to be ideal. This presumes that drugs can be developed that will discriminate between a particular oncoprotein and its corresponding normal protein in its context. This ideal condition is not achieved even with one of the best examples of targeted therapy in cancer, *i.e.* imatinib mesylate targeting the breakpoint cluster region (BCR)–Abelson murine leukemia viral oncogene homologue (ABL), BCR-ABL1, fusion protein in chronic myelogenous leukemia (CML). In fact, imatinib inhibits the kinase activities of both BCR-ABL and ABL, in addition to several other cellular kinases. Nevertheless, probably due to the high and specific dependence of CML cells on the BCR-ABL1 fusion protein, imatinib has a high therapeutic index for CML.

Table 2. WHO-recognized disease-defining abnormalities(6) in AML and their 'druggability'.

Fusions	'druggability'	Mutations	'druggability'
RUNX1-RUNX1T1	-	*NPM1*	-/+[4]
CBFB-MYH11	-	*CEBPA* (biallelic)	-
PML-RARA	+++[1]	*RUNX1* (provisional)	-
MLLT3-KMT2A	+/-[2]		
DEK-NUP214	-		
**GATA2, MECOM*	-		
RBM15-MKL1	-		
BCR-ABL1 (provisional)	+++[3]		

**GATA2* haploinsufficiency and *MECOM* (*EVI1*) overexpression;
[1]ATO+ATRA; [2]DOT1L-inhibitors, ongoing studies; [3]BCR-ABL1 tyrosine-kinase inhibitors; [4]ATO+ATRA (?), ongoing studies

Accordingly, it might be more difficult to develop drugs that directly inhibit oncoproteins that result from point mutations without affecting their normal counterparts. A second way to achieve enhanced cancer-cell selectivity would be to identify situations where the requirement for a particular target is enhanced in the context of a cancer cell compared with normal cells (the so-called 'context-driven therapeutic index'). In this case, the 'target' is not specific of the cancer cell being present also in the normal cell, but only the cancer cell, due to its new condition established as consequence of the mutational event, is dependent on it. This differential requirement might be because of intrinsic differences in the cells (e.g. differential genes expression) or extrinsic (e.g. differential cell interactions with the microenvironment)(8). Although the majority of drugs available today to treat cancer affect targets that are shared between normal cells and cancer cells, remissions of disease and even cure can be reached, suggesting that contextual differences between normal cells and cancer cells are therapeutically exploitable and worth to be explored. As already mentioned, with the increased understanding of the molecular events occurring during the development of AML, the number of potential targets for therapy has also grown. These targets can be placed into three broad categories: 1) the mutational events leading to AML; 2) cell surface antigens; 3) the adaptive, new context-driven nonmutational changes in the cancer cell given the initial mutational event (Figure 1). Although treatment strate-

gies targeting cell surface antigens in AML are an active field of research and are emerging as novel promising approaches to treat AML in the near future, I will focus my dissection on the other two categories of therapeutic targets, on the light of the most recent discoveries. *Mutational events as target of therapy in AML:* In maximizing the benefits of molecular therapeutics in cancer, it is necessary to have an understanding of the underlying molecular abnormalities and mechanisms involved. It is reasonable to think that drugs hitting cellular and molecular targets that are causally involved in the formation, growth, and progression of AML might be also active therapeutic agents. Considering the different AML-associated genetic lesions, classified accordingly to TCGA(2), unfortunately, the majority of mutational events are not "druggable" at the moment. These include the following categories: *Tumor suppressors (16% of cases)*, with *TP53, WT1, PHF6*; *Myeloid transcription-factors (22% of cases)*, with *RUNX1, CEBPA,* and other myeloid TFs; Chromatin-modifiers (30% of cases), which include *MLL-X* fusions, *MLL-PTD, NUP98-NSD1, ASXL1,* and other modifiers; *Cohesin-complex genes (13% of cases); Spliceosome-complex genes (14% of cases); NPM1 (27% of cases)*.Among the other categories, examples of either successful or at least promising targeted therapy which represent the object of an intensive clinical experimentation can be found: *Transcription-factor fusions (18% of cases):* This category includes, among the most frequent, *PML-RARA, MYH11-CBFB, RUNX1-RUNX1T1, PICALM-MLLT10.* Of these, only *PML-RARA* is 'druggable' using ATRA and ATO, which induce degradation of the oncofusion protein, allowing a cure rate of over 90% of APL patients harbouring this genetic lesion(5); *DNA-methylation–related genes (44% of cases):* This category includes, among the most frequent, *DNMT3A, DNMT3B, DNMT1, TET1, TET2, IDH1* and *IDH2.* Although different drugs with an epigenetic modulator action have become available for AML treatment, only for IDH1 and IDH2 oncoproteins, drugs directly affecting their activities have been developed. *IDH1/2 as target of therapy.* IDH1 and IDH2 are homodimeric NADP-dependent enzymes that, in the Krebs cycle, catalyse the oxidative decarboxylation of isocitrate to alpha-ketoglutarate (a-KG), with reduction of NADP+ to NADPH. IDH1 exerts its activity in the cytoplasm and peroxisomes, whilst IDH2 acts in the mitochondrial matrix. These enzymes are involved in the metabolism of glucose, fatty acids and glutamine and regulate the cellular redox status. Mutations of *IDH1* or *IDH2* genes occur in 15–20% of all AML and mostly cluster with CN-AML. They are not AML-specific, being detectable also in other either hematological or not hematological malignancies. However, their role in contributing to AML development has been established in animal models.

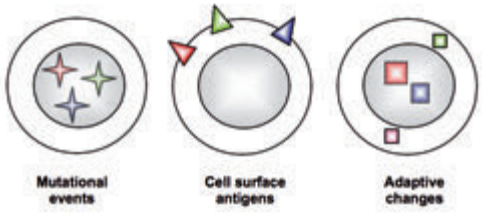

Figure 1. Categories of therapeutic targets.

Hence, they became of particular interest as therapeutic targets. *IDH1* mutations occur at the R132 codon, while *IDH2* mutations occur at the R140 or R172 codons and invariably alter the catalytic site reducing their affinity for isocitrate and conferring them the ability to convert the a-KG to 2-hydroxyglutarate (2-HG) which accumulates. As 2-HG is a competitive inhibitor of several -KG-dependent histone demethylases, its accumulation disrupts normal DNA methylation and arrest lineage-specific progenitor cell differentiation, providing the basis for leukemogenesis. Several pre-clinical studies targeting IDH demonstrated promising anti-leukemic effects. From these studies emerged AG-221 (enasidenib), as first-in-class selective small molecule IDH2 inhibitor. The results of a phase I/II multicenter trial investigating the role of AG-221 in AML with *IDH2* mutations suggest that enasidenib is safe and may improve survival in patients with relapsed or refractory (R/R) AML, yielding overall responses in 40.3% of patients (19.3% CR) and a median overall survival of 9.3 months(9). Interestingly, a differentiation syndrome similar to that one described in APL with ATRA has been reported in some patients. Additional trials with AG-120 in combination with conventional therapies and hypomethylating agents (HMA) are currently underway. Following these studies, enasidenib (IDHIFA, Celgene Corp.) received FDA approval for *IDH2*-mutated R/R AML on the 1st of August 2017. Also IDH1 inhibitors have shown promising results. The first-in-class IDH1-inhibitor is AG-120 (Ivosidenib) which gives in R/R AML an overall response rate of 33% (16% CR) with 6.5 month median duration of response (DiNardo et al, Abs#1070, ASH 2016). This drug is now being studied in multiple clinical trials alone or in combination with chemotherapy or HMA. It is expected that a similar expedited regulatory strategy that is being utilized for enasidenib for FDA approval will be applied also for AG-120. *Activated signaling genes (59% of cases):* Also in this category, which includes, among the most frequent mutated genes, *FLT3, KIT, KRAS/NRAS* and *PTP*s with other Tyr kinases and Ser–Thr kinases, major progresses in targeted therapy have been made in the recent years, particularly with FLT3 inhibitors. *FLT3 as target of therapy.* FLT3 is a receptor tyrosine kinase physiologically expressed as a monomeric transmembrane protein on early haematopoietic precursors. FLT3 is normally activated through interaction with its ligand (FLT3L) that promotes the dimerization and phosphorylation of the receptor and triggers several downstream signalling pathways promoting cell proliferation and pro-survival properties. *FLT3* mutations occur either as *FLT3*-ITD or *FLT3*-TKD (point mutations in the activation loop of tyrosine kinase domain) in about 30% of AML. Although ITD and TKD mutations appear late in the clonal evolution of AML, they remain critical for the survival of leukemic cells. Efforts to develop inhibitors of the mutated forms of the FLT3 receptor have led to successive generations of FLT3 inhibitors, many of which have been and are being used in clinical trials (i.e. midostaurin, sorafenib, quizartinib, crenolanib, and gilteritinib), with limited success as monotherapy. The drug that received a wide consensus and FDA approval on the 28th of April 2017 is midostaurin (RYDAPT, Novartis Pharmaceuticals Corp.), for the treatment of adult patients with newly diagnosed FLT3-positive AML in combination with standard chemotherapy. This was based on the results of the RATIFY trial(10), which showed higher either EFS (24.2% vs. 21.8%) than OS (51.4% vs. 44%) at 5 years when midostaurin was added to chemotherapy. "*Context-driven" target therapy in AML: targeting adaptive changes and new dependencies:* To develop a 'context-driven' targeted therapy requires a deep knowledge of the complex mechanisms mediating the oncogenic effects of a specific genetic lesion, as well as of the differential requirements between cancer and normal cells. Different players have been identified which are now targets of specific drugs. The most explored in clinical trials at the moment are listed below: *Epigenetic enzymes as target of therapy*. In AML, epigenetic gene silencing caused by DNA methylation is a potential target for demethylating (or hypomethylating) agents. These drugs do not target a specific genetic lesion, but, rather, they target the abnormal global methylation patterns newly established in AML cells. A promising new HMA is guadecitabine (SGI-110), a second-generation HMA which is resistant to degradation by cytidine deaminase and thereby with longer half-life as compared to other HMAs ensuring a more prolonged exposure of tumor cells to the active metabolite, decitabine. Results of first trials are promising, with 12% and 34% CR rates in R/R AML and treatment-naïve AML patients, respectively. Currently guadecitabine is in phase III development. *DOT1L as target of therapy*. The disruptor of telomeric silencing 1-like (DOT1L) and the bromodomain and extraterminal (BET) epigenetic reader proteins have been shown to play a role in the oncogenesis mediated by the MLL fusion proteins. *MLL* gene rearrangements are found in about 10% of adult AML and confer a poor prognosis. MLL fusion proteins have been shown to associate with the H3K79 histone methyltransferase DOT1L complex, which leads to the up-regulation of several genes directly involved in leukemogenesis (e.g., HoxA9 and MEIS1). Inactivation or inhibition of DOT1L have been demonstrated to inhibit leukemia development in animal models of *MLL*-rearranged AML. The DOT1L inhibitor pinometostat (EPZ-5676) was recently tested in clinical trials showing reductions in the methylation of target genes of the MLL fusion protein but with modest clinical activity. *BCL-2 as target of therapy*. One of the most frequent adaptive changes found in cancer cell is upregulation of antiapoptotic proteins. BCL-2 is anti-apoptotic protein frequently overexpressed in AML and playing an essential role in the maintenance and survival of AML cells, as well as in their chemoresistance. Targeting the BCL-2 family of proteins is an attractive option and small molecules targeting

the BH3 domain of BCL-2 proteins, also known as BH3-mimetics, have been developed to stimulate this essential apoptotic pathway. Venetoclax (ABT-199) received major consensus. Although pre-clinical and clinical studies indicated modest single-agent activity (19% response rate in R/R AML), combination trials with either low dose citarabine (LDAC) or HMAs are very promising (Wei et al, Abs#102, ASH 2016; DiNardo et al, Abs#327, ASH 2015). Early results from the phase Ib trial (NCT0220377) with venetoclax in combination with either decitabine or 5-azacytidine are encouraging, as CR/CRi was achieved in 16/22 (5 CR) patients with very good tolerability (DiNardo et al, Abs#327, ASH 2015).

Final remarks: It is really an exciting time in the field of both translational and clinical research in AML. Survival curves in AML patients are going up, not just because of supportive care, but also because we understand the disease better and define better the treatment strategy. Targeted therapy is rapidly growing with different new drugs already available. The challenge is going to be the proper use of these agents, and in a carefully designed rational way. The future will be about combinations and collaborations, as we are dissecting cytogenetic, molecular genetic subsets of AML into smaller groups. However, still many progresses must to be done to discover ways to target the genetic lesions that are disease-defining and as such recognized by the WHO classification. In fact, if we focus on the WHO-recognized AML entities, we realize again that only APL with *PML-RARA* rearrangement is receiving a specific targeted therapy (Table 2). The success of ATRA+ATO therapy relies most likely on its ability to degrade the oncofusion protein produced by the underlying disease-defining genetic lesion.We believe that if we could hit either RUNX1-RUNX1T1, CBFB-MYH11 fusion oncoproteins or NPM1 mutant leukemic protein, for example, we could achieve similar results as those of APL therapy today. By different screening approaches, through collaborative science and intense focus on each individual AML subtype, we can continue to develop therapies for specific targets and have a smaller fraction of patients treated with the standard '7+3' approach that has defined AML therapy for decades.

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THE ROLE OF CONVENTIONAL AND NEW COAGULATION ASSAYS FOR MONITORING DIRECT ORAL ANTICOAGULANTS: INDICATIONS AND LIMITS

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Introduction: Currently approved direct oral anticoagulants (DOACS) include dabigatran (FIIa inhibitor), along with rivaroxaban, apixaban and edoxaban (FXa inhibitors). Laboratory monitoring of new direct oral anticoagulants is not routinely recommended; however, it may become necessary in particular, clinical settings like urgent surgical procedures, life-threatening bleedings, renal or hepatic failure, recurrent thrombosis during treatment, overdose. Actually, measurements of plasma DOAC levels may be occasionally needed for all patients, thus "ideal" tests should be sensitive, accurate as well as easy to perform, rapid and ubiquitous. In 2013, the Scientific and Standardization Committee (SSC), through its subcommittee on Control of Anticoagulation, of the International Society on Thrombosis and Haemostasis defined the recommended methods for measuring DOACs [1]. However, clinical and laboratory evidences deriving from the growing use of DOACs in the last years need to be updated. Primary aims of the current report were to analyse available data on laboratory assays for qualitative and quantitative assessment of DOACs and identify the most adequate tests for each DOAC.

Conventional coagulation tests Prothrombin Time and Activated Partial Thromboplastin time: Conventional coagulation assays, including prothrombin time (PT) and activated partial thromboplastin time (APTT), are widespread and available round the clock in every laboratory. Normal PT may be able to rule out excessive levels of two anti-Xa DOACS, rivaroxaban and edoxaban. However, PT does not allow any evaluation for typical on-therapy levels of both drugs. PT is usually not sensitive to apixaban and it varies based on the thromboplastin reagent adopted, thus a normal PT value is not able to exclude excessive levels of apixaban. APTT assay is not recommended for measurement of factor Xa inhibitors due to its low sensitivity. For the Factor IIa inhibitor dabigatran, a normal APTT excludes excessive plasma levels of the drug, but is not able to rule out typical on-therapy dabigatran concentrations. In most situations, PT is not enough sensitive to dabigatran. Overall, several factors limit the use of either PT or APTT for assessing DOACs concentration, including reagent, calibrators and instrumentations adopted. Prolonged test results may not be attributed only to drug levels, but also to combined factor deficiencies or inhibitors. However, any prolongation of the APTT (and PT) in patients under DOAC treatment should be first attributed to drug effect until proven otherwise by more specific assays. Table 1 summarizes adequacy of each laboratory coagulation assay for each new DOAC. *Thrombin time:* Thrombin time (TT) can give general information related to the presence of high concentrations of dabigatran; a normal TT excludes clinically relevant levels of this drug. The dilute thrombin time (dTT) is a modified TT test where patient's sample is preliminary diluted with a buffer. dTT can be easily performed with most of the available automated instruments, although for some analysers diluted samples may need to be manually prepared. dTT measured with Hemoclot® is able to better quantify dabigatran across a broad range of concentrations, but its availability is limited. *Dilute Russell viper venom time:* Dilute Russell viper venom time (DRVVT) better mirrors drug plasma levels of DOACs, it may be thus appropriate for a fast estimation of drug levels, after an accurate calibration with drug-specific reagents. DRVVT with added phospholipids has been proposed as a universal test for monitoring DOACs [2] because it allows the measurement of on-therapy plasma concentrations. *Ecarin Clotting Time and Ecarin chromogenic assay:* Clot based or chromogenic Ecarin testing show a good correlation with dabigatran levels measured by the gold standard method of mass spectrometry. Ecarin Clotting Time (ECT) may be easily performed with most coagulation analysers, particular care must be however given to avoid carryover effects between cycles. Ecarin based assays have been in general less well standardized than dTT methods. Chromogenic Ecarin assays (ECA) are not approved worldwide, thus their use may be limited. *Chromogenic anti FXa assay:* Factor Xa inhibitors may be quantified with anti-Xa assays, using drug-specific standards for calibration. Drug activity measurement by chromogenic anti-Xa assay is preferred against PT, APTT or dilute Russell viper venom time (DRVVT) because it gives a more precise and linear measurement of anti FXa drugs with lower bias [3]. To improve assay reliability, calibration with standard concentrations verified with LC-MS/MS method may be adopted. *New coagulation assays High-performance liquid chromatography-tandem mass spectrometry:* High-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) assays, adopted to measure plasma drug levels during registration trials, allow a direct dosage of anticoagulant plasmatic concentrations of rivaroxaban, apixaban, edoxaban and dabigatran. HPLC-MS/MS shows a higher selectivity than coagulation-based assays; it is thus considered the gold standard method for DOACs plasma measurement. HPLC-MS/MS is characterized by a high specificity, sensitivity and reproducibility, however, it is quite complex to perform and test results may be influenced by the lack of standardization and well defined reference standards [4]. Drug metabolites may also interfere with HPLC-MS

measurements. *Global haemostatic tests and thrombin generation assays:* Global haemostatic assays (GHA) like thromboelastography (TEG) and thrombin generation, are currently under study for anti-IIa and anti FXa anticoagulation monitoring. They are currently not enough specific to correlate their abnormal results with DOACs levels. GHA thus require further standardization to be accurately adopted in this setting. A recently published research showed a highly significant correlation of a novel, thromboelastometry-based assay, the CT EcaTEM, over a wide range of plasma levels of dabigatran, comparable to the Hemoclot® assay currently recognized as the gold standard for the measurement of dabigatran plasma levels [5]. On the contrary, TEG accuracy is limited for apixaban and rivaroxaban, mostly in cases of low drug plasma concentrations. At rotational thromboelastogram (ROTEM) in patients under dabigatran, increased R times and clot formation time were observed [6]. In emergency settings, thromboelastography may be a useful tool to identify the anticoagulant effects secondary to DOAC therapy and drive bleeding management decisions or drug reversal [5,7]. *Point of care tests:* The so-called "point of care" testing, including activated thrombin time and prothrombinase-induced clotting time (PiCT), have a poor correlation with therapeutic levels of DOACs and conflicting results have been reported by the available studies.

Table 1. Adequacy of conventional and new coagulation assays in monitoring new direct oral anticoagulants.

Laboratory assay	*Dabigatran*	*Rivaroxaban*	*Edoxaban*	*Apixaban*
PT	May be adequate	May be adequate	May be adequate[1]	Not adequate[2]
APTT	May be adequate[3]	Not adequate	Not adequate	Not adequate
dTT	**Adequate**	Not adequate	Not adequate	Not adequate
ECT	**Adequate**	Not adequate	Not adequate	Not adequate
DRVVT	Partially adequate	Partially adequate	Partially adequate	Partially adequate
*Anti-Xa assay**	Not adequate	**Adequate**	**Adequate**	**Adequate**
HPLC-MS°	**Gold standard**	**Gold standard**	**Gold standard**	**Gold standard**
GHA	May be adequate	May be adequate	May be adequate	May be adequate
TG	Not adequate	Not adequate	Not adequate	Not adequate

*Clotting Time; ECA= Ecarin chromogenic assay; DRVVT= Diluted Russel Viper Venom; HPLC-MS= High-performance liquid chromatography-tandem mass spectrometry; GHAs=Global Haemostatic Assays; TG=Thrombin Generation. 1. Shown less adequate than for rivaroxaban; 2.Completely insensitive to apixaban plasma levels; 3. Reagent and instruments may be appropriately settled for drug monitoring due to reported wide variability; *Chromogenic assay; °Allows drug measurement.*

Notes: Adequacy of each assay may be influenced by the type of reagents/instruments adopted and plasma sampling/time interval from drug intake, further studies are needed to standardize assays. Ecarin based clotting tests are less standardized than dilute thrombin time for patients under dabigatran.

Conclusions: In summary, anti FXa chromogenic assays testing are considered the best options for Apixaban, Edoxaban and Rivaroxaban, while diluted TT and ECT are optimal for Dabigatran. HPLC-MS/MS allow an accurate measurement of drug plasma levels. When DOAC measurement is clinically needed, a timed sample collection (expected peak or through) should be preferred. Univocal recommendations cannot however be drawn as the most appropriate tests are related to several variables including: the type of DOAC, clinical needs, emergency or elective settings, assay availability [8].Results of any qualitative or quantitative DOAC measurement should be interpreted taking into account the patient clinical history and last known dose intake. Laboratories that perform quantitative measurements of DOACs should be involved in external quality assurance programs to improve result accuracy and support the definition of high-quality and international reference standards.

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NOVEL INSIGHTS IN DIAGNOSIS AND TREATMENT OF GVHD

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Pathofisiology: Allogeneic haematopoietic stem cell transplantation (SCT) is the only curative option for many haematological malignancies. However, the development of graft-versus-host disease (GVHD) limits allo-SCT success. The classic description of **acute GVHD** (aGVHD) pathophysiology begins with activation of host antigen-presenting cells (APCs) by danger signals expressed on damaged tissues (damage-associated molecular patterns [DAMPs]) and/or pathogens (pathogen-associated molecular patterns [PAMPs], eg, lipopolysaccharide). Activated host APCs then present host antigens to donor T cells, leading to alloactivation and inflammatory cytokine release. These inflammatory cytokines recruit and induce proliferation of additional immune effector cells, automaintaining alloreactive tissue injury and inflammation. Several non-genetic triggers of aGVHD have been identified, predominantly danger signals: DAMPs and/or PAMPs. DAMPs include extracellular matrix components, adenosine triphosphate, and uric acid. Heparan sulfate, a component of extracellular matrix and endogenous Toll-like receptor agonist, which can promote alloreactive T-cell responses, is elevated in both murine and human GVHD, but not after conditioning. ATP released by dying cells can also induce inflammatory responses, and ATP neutralization or blockade of its receptor on immune cell subsets, P2X7R, reduced experimental GVHD. Uric acid can also act as a DAMP, leading to NLRP3 inflammasome-mediated interleukin-1b production, a key cytokine involved in aGVHD pathophysiology. In addition to endogenous danger signals, bacterial and viral PAMPs can both contribute to the inflammatory milieu. The classic target organs of aGVHD are the skin (severity ranging from maculopapular rash to erythroderma and bullae formation), the gastrointestinal tract (resulting in nausea, vomiting, abdominal cramps, and/or diarrhea) and the liver (resulting in hyperbilirubinemia, jaundice, and/or elevated trans- aminases). The hematopoietic system can also be targeted. Endothelium, lungs, and other organs can also be targeted, although skin, gut, and liver involvement are the only organs scored in the current grading system.

Chronic graft-versus-host disease: (cGVHD) remains the major cause of morbidity and non-relapse mortality after SCT. Progress in improving cGVHD prevention and therapy has been hindered by complexities in cGVHD diagnosis and lack of uniform treatment response criteria. cGVHD typically manifests with multiorgan damage and historically has been defined temporally as GVHD occurring later than 100 days post-SCT. The commonly seen diagnostic features, according to the National Institutes of Health (NIH) consensus criteria, include skin lichen planus–like lesions or sclerosis, bronchiolitis obliterans (BO), and oral lichen planus–like lesions. Esophageal webs and strictures and muscle or joint fasciitis are also diagnostic. Importantly, these diagnostic features can be seen before day 100 and may occur simultaneously with features commonly seen in aGVHD (eg, macular-papular rashes, weight loss, diarrhea, and hepatitis). Thus, cGVHD can occur as a continuum in time with clinical features that are distinct from, but not mu-

tually exclusive with, those seen in aGVHD. Transplanted mice receiving pre-SCT conditioning regimens, typically radiation-containing, can progress through a continuum of aGVHD to cGVHD which can evolve over time. In fact, autoreactive T cells can coexist with or emerge from alloreactive T cells. *A noteworthy distinction between the pathology of aGVHD and cGVHD* is the typical tissue inflammatory T-cell infiltrate and destructive features of aGVHD and the relatively acellular, fibroproliferative findings in cGVHD. In particular, scleroderma, BO, and fibrosis in liver, gastrointestinal tract, salivary glands, and tongue can be seen in cGVHD mouse models. The target organ injury observed in both aGVHD and cGVHD is a consequence of the coordinated interplay between multiple cellular and molecular immune mediators that is dependent on the presence and function of donor graft T cells. Following SCT, tissue injury and inflammation characterized by pro-inflammatory cytokine release (eg, tumor necrosis factor [TNF], interleukin-6 [IL-6], and IL-1) is initiated by the conditioning regimen that would be common for both aGVHD and cGVHD, as both diseases can develop in patients who receive the transplantation procedure. These cytokines, together with luminal damage-associated molecular patterns and pathogen-associated molecular patterns, released from damaged gut tissue and the microbial luminal contents, result in the activation of antigen-presenting cells (APCs). Activated APCs then prime naive donor T cells and preferentially drive T-helper 1 (Th1)/T-cytotoxic 1 (Tc1) and Th17/Tc17 differentiation and expand T-effector cells, which can mediate target tissue GVHD, including the thymus, as well as the skin, liver, gastrointestinal tract, and lung, likely predisposing to cGVHD later after SCT. aGVHD is generally defined as a Th1/Th17 paradigm, which results in extensive tissue destruction characterized by apoptosis. Nevertheless cGVHD and aGVHD may share initiating mechanisms; for example, Th17/Tc17 cells have been shown to cause either aGVHD or sclerodermatous cGVHD. However, although donor natural killer cells (NK), T-regulatory (T-regs) cells, regulatory B cells, and macrophages play important roles in dampening both aGVHD and cGVHD, the role of B cells in controlling aGVHD pathogenesis at least in murine models is controversial, while in cGVHD, there is an interplay between donor T cells and donor B cells for disease pathogenesis. Today, the backbone of most T-cell replete conventional **GVHD prophylaxis regimens** includes calcineurin inhibitor plus MTX or mycophenylate mofetil or post-SCT Cyclophosphamide. Alternative methods of GVHD prophylaxis include T-cell depletion (CD34 positive selection or through T-cell subset depletion methods targeting CD3, alpha-beta T cells, or in vivo, via administration of antithymocyte globulin or alemtuzumab). The results of these studies have prompted new multicenter comparative clinical trials. First, a phase 1/2 study of maraviroc, a CCR5 inhibitor that blocks lymphocyte chemotaxis while preserving effector functions, demonstrated very low rates of grade II-IV acute GVHD (15%) and no visceral GVHD at day 100 post-HCT in 35 evaluable patients. Next, addition of bortezomib, an NF-kB–inhibiting immunomodulator, given on days 11, 14, and 17 in addition to standard tacrolimus and MTX in mismatched unrelated donor HCT, resulted in a very low incidence of grade II-IV aGVHD (22%), considering the mismatch donor source. Finally, a phase 1/2 study of vorinostat, used for its tolerogenic effects on APCs and apoptotic effects on alloreactive T cells, given from 10 days prior to transplant through day 100 in addition to the backbone of immunosuppression, showed promising data.

Modern treatment of acute and chronic GVHD: The standard treatment of patients with **aGVHD,** requiring systemic therapy is corticosteroids at a daily dose of 2 mg/kg, although there appears to be little detriment to a lower daily dose of 1 mg/kg for overall grade 1-2 aGVHD, thereby sparing some side effects of high-dose steroids for milder disease. The determination of steroid-responsive or refractory disease should be made within a few days of initial therapy, recognizing nonresponse after 7 to 10 days and progression even sooner if the patient is clearly worsening 3 to 4 days after the start of high-dose steroids. The optimal duration of steroid therapy is unknown, but should be limited if possible to avoid side effects of long-term administration. While steroids remains the first line of intervention, *there is no standard second-line treatment for steroid-refractory aGVHD (SR-aGVHD)*; several novel therapeutic options are being investigated. Currently, no agents are approved by FDA or EMA for either prevention or treatment of aGVHD. Formal precedents establishing a comparative basis for assessing the efficacy and safety of new investigational agents are still lacking. Anti-thymocyte globulin or extracorporal photopheresis (ECP) have shown some activity, but none has been established as a standard salvage therapy for SR-aGVHD. Mesenchymal Stem Cells (MSCs) are multipotent non-hematopoietic stem cells that can differentiate into various tissues, repair injured tissues and modulate allogeneic immunoreactions. One of the most pronounced characteristics of these cells are their immunosuppressive properties. The first case of severe SR-GVHD successfully treated with MSC was described in 2004. The largest was a multicenter phase I/II study of 55 European patients, published in 2008 on behalf of EBMT. More then fourteen studies have been reported in about 200 patients, showing response rate varying from 0% to 100%. MSCs have been used both for the prophylaxis of aGVHD and the treatment of patients with SR-aGVHD, at a dose of 1.0 106/kg body weight. However, there are few data regarding the efficacy and safety of MSC for cGVHD. There is only one published report that demonstrated a temporary response of MSC in one patient with liver cGVHD and some cases report in BOS. In conclusion MSCs are promising in GVHD, but the encouraging results still wait to be confirmed in randomized studies. Alemtuzumab is a monoclonal antibody inducing a strong T-depletion highly effective in preventing GVHD; moreover at least 8 retrospective series showed a relevant response rate in SR- aGVHD, but the infectious complications rate was not negligible. CD 30 Ag plays an important role in aGVHD; indeed activated T cells highly express CD30; in a preliminary phase 1 study Brentuximab-Vedotin was relatively safe at the MTD of 0.8 mg/kg and showed a remarkable activity in SR-aGVHD, with an overall response rate of 37.5%. With a median follow-up of 12.9 months, 9/24 patients were alive. Another relatively novel approach for SR-aGVHD is represented by targeting the mediators and amplifiers of the tissue damage with anti-cytokine Abs (ie anti-Il2 antibodies; anti-Il-6 antibodies and anti-TNF antibodies); however no randomized controlled studies have been conducted in order to confirm the preliminary promising results coming from phase 2 studies.

Interaction between selectin and their ligands modulates the trafficking of alloreactive T-cells represents another novel approach for SR-aGVHD; P-selectin is costitutively expressed on vascular endothelium of the skin and bone marrow, and can be induced in other endothelial cells during inflammation. Blockade of selectin/ligand axis impairs homing of alloreactive T-cells. The $\alpha4\beta7$ integrin is expressed on lymphocytes, NK cells, mast cells and mediate lymphocyte binding to MAdCAM-1. Vedolizumab is a monoclonal antibody which specifically antagonizes $\alpha4\beta7$ gastrointestinal integrin and has been successfully tested in inflammatory bowel disease refractory or intolerant to a TNF-α antagonist; and recently 7 patients receiving this Ab for severe gut SR-GVHD, exhibited dramatic responses. It is reasonable to believe that Vedolizumab will be primarily effective in intestinal GVHD and should probably not be used as a single agent for aGVHD affecting other districts. CD26 is another possible target to contrast the activated T-cell homing. This receptor is highly expressed on T cells migrating to inflamed tissues. Experimental data show that inhibition of CD26 blocks the migration of T cells across the endothelial wall and that preserves pancreatic islet transplants from T cell attak in mice. Preliminary data from Bacigalupo et al showed a dramatic efficacy of the anti-CD26 antibody in patients with severe SR-aGVHD. *The JAK– STAT pathway* is involved in the signaling function of many inflammatory cytokines and this ultimately impacts differentiation of key effector cells in both acute and cGVHD. A retrospective survey has been recently reported in 95 patients, treated with ruxolitinib for SR-aGVHD and SR-cGVHD. Patients were classified as having SR-aGVHD (54, all grades III-IV) or SR-cGVHD (41, all moderate or severe). The overall response rate was 81.5% in SR-aGVHD, including 25 complete responses (46.3%), while for SR-cGVHD the ORR was 85.4%. The 6-month-survival was 79% and 97.4% for SR-aGVHD and SR-cGVHD, respectively. Cytopenia and cytomegalovirus-reactivation were observed in both SR-aGVHD (55.6% and 33.3%) and SR-cGVHD (17.1% and 14.6%) patients. Up today ruxolitinib may constitute a promising new treatment option for SR-aGVHD and SR-cGVHD that should be validated in prospective trials: indeed two phase 3 studies, in aGVHD and cGVHD are currently ongoing to confirm these data both in aGVHD and in cGVHD. Today's **standard of care in the treatment of cGVHD** has been unchanged over the past several decades. First line treatment of cGVHD continues to be corticosteroids, with or without a calcineurin inhibitor. In terms of second-line therapy, there is no standard of care, and there exist a number of small, non-randomized studies or case series that have reported various therapies, none of which are known to be superior over another. Standard drug development pathways for other diseases have not been feasible in cGVHD for several reasons,: cGVHD is a rare, orphan disease, where the dismal prognosis makes difficult to conduct a

conventional phase 3 study; moreover although a number of animal models for cGVHD exist, none captures all of the manifestations. Among the several new drugs tested in SR-cGVHD, recently imatinib emerged as a potent dual inhibitor of both transforming growth factor-b (TGF-b) and platelet-derived growth factor receptor (PDGF-R) pathways; these 2 cytokines are both involved in the fibrogenic and inflammatory processes of several fibrotic diseases, Moreover, imatinib inhibits T-cell proliferation, as suggested by clinical improvement in patients with chronic myelogenous leukemia and concomitant autoimmune diseases receiving imatinib treatment. After 6 months, intention-to-treat analysis of 39 patients with severe SR-cGVHD, who received Imatinib, showed 14 partial responses, 4 minor responses with relevant steroid sparing (46%) according to Couriel criteria, and PR (51.3%), as per the National Institutes of Health (NIH) criteria. The second generation TKI, Nilotinib has been recently evaluated in a multicenter phase I-II study, showing a good safety profile and a 71% PFS at 24 months. The proteasome inhibitor bortezomib has immunomodulatory properties with the ability to selectively deplete proliferating alloreactive T lymphocytes, reduce T-helper Type 1 cytokines, and block antigen presenting cell activation. Bortezomib may also spare regulatory T cells that may be relevant in GVHD control. In a mouse model of cGVHD, bortezomib ameliorated cutaneous lesions, associated with a reduction in total numbers of germinal center B cells and lower B-cell activating factor gene expression levels in cutaneous tissues. Lymphoma-bearing mice receiving allogeneic SCT with bortezomib preserved graft- versus-tumor effects. Basing on this background a clinical trial in patients with extensive SR-cGVHD, showed marked clinical improvement, associated with reductions of peripheral B cells and minimal toxicity. *CD4 CD25 Foxp3 Tregs* comprise 5% to 10% of circulating CD4 T cells, suppress autoreactivity, and control innate and adaptive immune responses. Treg impairment is associated with loss of tolerance, autoimmunity, and cGVHD. Adoptive transfer of Tregs can ameliorate GVHD in preclinical models. An alternative strategy to augment Tregs *in vivo* is represented by the administration of low physiologic doses of IL-2 which is critical for Treg development, expansion, activity, and survival. In a phase 2 study low-dose IL-2 was safe and effective in SR- cGVHD, with durable disease control. Twenty of 33 evaluable patients (61%) had objective PR and during 2 years of extended IL-2 therapy, clinical and Treg immune responses persisted, while Treg:Tcon ratio gradually normalized. Recently new agents *targeting T-cell migration* from lymph nodes to GVHD target organs have been developed. In particular, sphingosine 1-phosphate (S1P) receptor modulators have been studied in multiple sclerosis and preclinical work has been done in murine models of sclerodermatous cGVHD. Interaction between S1P and its receptors on lymphocytes has a central role in the process by which lymphocytes exit lymphoid organs and enter the circulation. S1P receptor modulators block this interaction, leading to reduction of circulating peripheral lymphocytes and subsequent recruitment to sites of inflammation. FTY720 (fingolimod), is an S1P inhibitor that demonstrated reduction in GVHD in a haploidentical murine model, but allowing a GVL effect, suggesting that fingolimod is able to limit the graft-versus-host response to the lymphoid tissue. Ponesimod is a second-generation S1P receptor modulator that is specific for the S1P1 receptor, which in particular has a primary role in regulation of T-cell trafficking, including egress of mature T cells out of lymph nodes after immune activation. Ponesimod also leads to rapid and reversible reduction in circulating lymphocytes, and again, other immune cells are not impacted. In cGVHD the high levels of the cytokine *B-cell- activating factor (BAFF),* which enhances B-cell proliferation and survival, may contribute to a lack of B-cell tolerance. An increase BAFF to B-cell ratio has been demonstrated in patients with cGVHD, like in patients with autoimmune diseases. Basing on these observations new treatments targeting B cells have been introduced, using agents originally developed for treatment of B-cell malignancies. Rituximab, the anti-CD20 monoclonal antibody, which is the backbone of many B-cell malignancy treatment regimens, has been tested with positive results in cGVHD. There has recently been a surge of development of novel agents for the treatment of B-cell malignancies by *inhibition of key tyrosine kinases* involved in the B-cell receptor signaling pathway; these are now being considered for treatment of cGVHD. One of the first agents in this area is ibrutinib, a BTK/ITK inhibitor approved for treatment of CLL, mantle cell lymphoma and Waldenstrom's macroglobulinemia. Ibrutinib acts as an inhibitor of both BTK (one of the proteins that has a key role in B-cell receptor signaling, which is increased also in cGVHD), as well as ITK, a protein involved in Th2 and Th17 receptor signaling. Thus, ibrutinib can impact multiple mechanisms of cGVHD pathogenesis. A multicenter Phase 1b/2 study in patients with SR-cGVHD showed that ibrutinib is safe, with the most common adverse events being those that are common with this agent in other patient populations (fatigue, diarrhea, bruising, nausea, and mucositis). Most importantly in 42 evaluable patients, the overall response rate was 67%, with long lasting responses. Ibrutinib recently received FDA breakthrough therapy designation and orphan drug designation for the treatment of cGVHD. Syk, is another key target; indeed Syk inhibition, using the agent *fostamatinib,* led to apoptosis and prevention of hyperresponsiveness in human cGVHD B cells as well as reduction in sclerodermatous disease and reversal of tissue damage in mouse models. Finally *non-lymphocyte targets* are emerging for potential therapies in chronic GVHD. One of these targets, neutrophils, is being evaluated in patients with BOS, the manifestation of cGVHD in the lungs. AZD9668 is an oral, selective Neutrophil Elastase inhibitor initially developed in inflammatory lung diseases. Preclinical studies in murine models of acute lung injury/acute smoke models showed that this agent prevented lung injury and reduced the inflammatory response. This agent has been studied clinically in patients with COPD, cystic fibrosis and bronchiectasis, and has been shown to be extremely well tolerated. Hedgehog inhibitors are another class of agents targeting non- lymphocytes in the treatment of cGVHD. Treatment of mice with the hedgehog inhibitor LDE223 prevented development of clinical and histologic features of sclerodermatous chronic GVHD.

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POLYCYTHEMIA VERA AND PRIMARY MYELOFIBROSIS: TOWARDS A PERSONALIZED THERAPY

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Multistep approach to personalized medicine in myeloproliferative neoplasms: Personalized (precision) medicine (PM) is a medical approach in which treatment strategies are tailored to the individual patient, based on the evaluation of predictive biomarkers for disease severity and responses. In 2005, the discovery of the role of the V617F mutation in the pseudo-kinase domain of the *JAK2* gene in the pathogenesis of myeloproliferative neoplasms (MPNs) provided a unified genetic basis for these neoplasms including Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Myelofibrosis (MF). The subsequent identification of somatic mutations in *MPL* and *CALR* genes, and the detection of other subclonal myeloid mutations (mainly, *ASXL1, SRSF2, EZH2, IDH1/2*) improved diagnostic and prognostic accuracy, leading to the development of specific inhibitors of JAK2 and introducing MPNs into the arena of precision medicine(1). An accurate diagnosis represents the basis for a personalized management of PV and MF. The 2016 World Health Organization (WHO) criteria have upgraded bone marrow histology as major diagnostic criterion in both diseases, emphasizing the distinction between *JAK2*^{V617F}-positive ET and PV and

recognizing early-PMF as a separate entity(2). Diagnosis is further implemented by individual assessment of the burden of the disease (e.g: symptoms, cytopenias, splenomegaly and comorbidities). Secondly, prognostic risk scores (reviewed by Guglielmelli *et al* in this issue) stratify patients in subgroups at different risk of thrombotic/hemorrhagic events, disease progression, and early death. Thirdly, different therapies are assigned to each risk category. In this review, we will describe the key-points of personalized therapy in PV and MF.

Personalized medicine in PV: Polycythemia Vera (PV) is the most frequent among classical MPN, with an incidence of 0.4-2.8 per 100 000 person-year. In the 2016 revision of the WHO classification, the diagnostic criteria of PV have been substantially modified by lowering the hemoglobin (Hb) or hematocrit (Hct) thresholds (Hb 16.5 g/dl, Hct 49% in men; Hb 16 g/dl, Hct 48% in females) and by upgrading bone marrow histology as major diagnostic criterion. These changes were made in order to distinguish *JAK2*-positive ET from the so-called "masked PV" and to assess the grading of marrow fibrosis found to correlate with the probability of fibrotic transformation(3). PV is characterized by absolute erythrocytosis, reflecting a clonal stem cell disorder, variably associated with leukocytosis and/or thrombocytosis, hepatosplenomegaly and systemic symptoms including vasomotor disturbances and aquagenic pruritus. PV is also associated with a high risk of thromboembolic events, and transformation to myelofibrosis/acute leukemia. Median survival for high-risk PV patients has been reported to be 10.9 years, with a 10-year projected rate for leukemic transformation and transformation into PPV-MF below 5% and 10%, respectively. Risk factors for reduced survival include advanced age, leukocytosis and previous venous thrombosis. Older age, leukocytosis, high *JAK2*^{V617F} allele burden, large splenomegaly and marrow fibrosis grade ≥1 are associated with increased risk of PPV-MF. The cumulative incidence of thrombosis ranges from 2.5 to 10.9% patient-year. Operationally, PV patients are stratified according to their likelihood of recurrent thrombosis. The risk score includes two risk categories: high-risk (age >60 years or thrombosis history) and low-risk (absence of both risk factors). In case of concomitant extreme thrombocytosis (platelets >1000x10^9/l), an acquired von Willebrand syndrome (avWS) may increase the risk of bleeding. Treatment is tailored to individual patients according to their risk of thrombosis, in order to: 1) prevent cardiovascular complications; 2) reduce the burden of symptoms; 3) delay or avoid progression to PPV-MF (Figure 1).

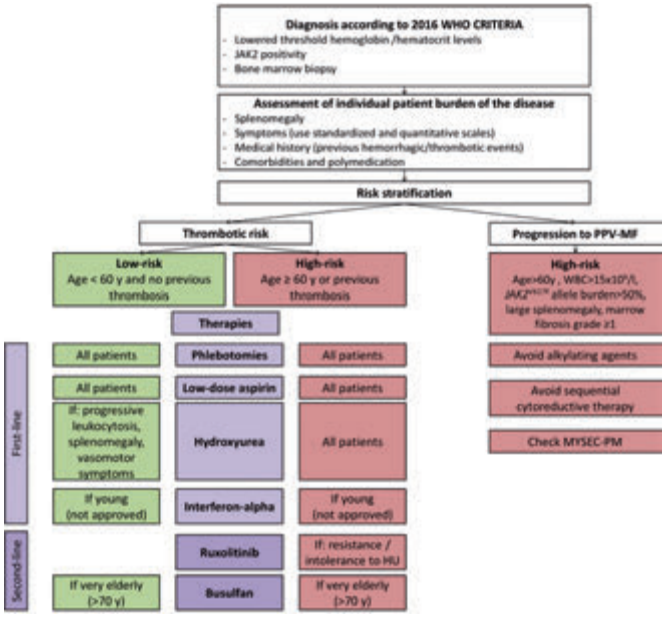

Figure 1. Personalized medicine in PV. Cardiovascular risk factors should also be aggressively managed in all patients.

Treatment of low-risk PV: The cornerstone of treatment of all-risk PV is the control of hematocrit (Hct) by phlebotomy. The optimal target of hematocrit (Hct) levels for reducing vascular events was recently clarified by a multicentre, randomized clinical trial (CYTO-PV), that randomly assigned 365 PV patients, irrespective of risk category and treatment (phlebotomy, hydroxyurea, or both), to a target level of less than 45% or 45%-50%. Patients in the higher hematocrit level had a 4-times increased rate of death from cardiovascular events in comparison to those maintained at less than 45%. Consequently, the Hct should be maintained strictly below 45% to efficiently reduce the risk of thrombotic events. Furthermore, the risk of thrombosis was significantly increased in patients with WBC count >11×10^9/L: therefore, cytoreduction should also be considered in low-risk patients with progressive leukocytosis(4). Low-dose aspirin is the second cornerstone of PV therapy. In the European Collaboration on Low-dose Aspirin in Polycythaemia Vera (ECLAP) study, a large European double-blind, placebo-controlled, randomized trial, baby aspirin was found to significantly reduce the primary combined end point, including: cardiovascular death, non-fatal myocardial infarction, non-fatal stroke and major venous thromboembolism. Aspirin may also be effective in alleviating vasomotor symptoms; in case of aspirin-resistant symptoms, cytoreduction may be considered. The use of low-dose aspirin requires caution in cases of extreme thrombocytosis that may induce an avWS with bleeding diathesis. In these cases, the study of vWF function (eg, ristocetin cofactor activity, vWF:RCoA) should be recommended and the start of cytoreductive therapy should be considered on individual basis. Notably, the correction of cardiovascular risk factors (smoking, overweight, hypertension, dyslipidemia, diabetes) should be aggressively pursued in all patients regardless of risk category(5).

Treatment of high-risk PV front-line: High-risk PV patients should receive cytoreductive therapy together with phlebotomy and low-dose aspirin. According to the European LeukemiaNet (ELN) recommendations, hydroxyurea (HU) and Interferon-alpha (IFN-) are recommended as first-line treatments for high-risk patients.(5) HU is an inhibitor of DNA synthesis via inhibition of the enzyme ribonucleotide reductase. Several studies including the phase II PV Study Group reported superior outcomes in reducing thrombotic events with HU when compared with phlebotomy alone. A potential long-term leukemogenic risk associated with prolonged use of HU has never been demonstrated, while a sequential use of cytoreductive therapies was found to correlate with increased incidence of acute leukemia. Therefore, alternative approaches to HU should be reserved to younger patients (<40 years) and those previously treated with other myelosuppressive agents. Recombinant Interferon-alpha (IFN-alpha) has antiapoptotic, antiproliferative, and immunomodulatory properties and has been used in PV for over 20 years, but is not approved yet. Results from the PROUD PV trial, a multicentre prospective study comparing peg-prolin IFN-alpha2b *vs* HU, showed comparable hematological control in both arms, with reduced hematological toxicity in the IFN arm (GisslingerH *et al*., 2016, Oral communication at ASH). However, the interim analysis of the MPDRC-112 study (NCT01258856) comparing peg-IFN-alpha2a *vs* HU failed to prove a superiority of IFN-alpha on achieving molecular and histological remissions (Mascarenhas JO *et al*., 2016, Oral communication at ASH).

Treatment of high-risk PV second-line: Around 20% of PV patients treated with HU have an inadequate response or unacceptable side effects. Notably, the occurrence of cytopenias during HU therapy predicts worse outcome. The advent of targeted therapy with JAK-inhibitors, in particular ruxolitinib, has extended the spectrum of agents that can be used as second or third line in PV. Ruxolitinib was approved for the treatment of inadequately controlled PV based on the RESPONSE and RESPONSE-2 phase 3 trials(6). These studies compared ruxolitinib to standard therapy in cohorts of PV patients resistant or intolerant to HU. In the ruxolitinib arm of the RESPONSE trial, 60% and 38% of patients showed either hematocrit control or spleen volume reduction, respectively, with 21% achieving both endpoints. Symptoms in 49% of patients treated with ruxolitinib (5% in the control arm) significantly decreased. Leukocyte count also decreased in the ruxolitinib arm. Most of the adverse effects reported in the RESPONSE study were grade 1 or 2, mainly headache, diarrhea, and fatigue. However, a two-fold increase in non-melanoma skin cancer was observed in the ruxolitinib arm, together with increased incidence of infectious complications.

Unmet clinical needs & unanswered questions. Is it possible to avoid disease progression and improve survival? As shown by the MPN landmark study, the main goal of therapy for PV patients should be to slow/delay disease progression. However, current therapies aim to decrease the thrombotic risk and do not directly address the issue of PV transformation. In the long-term follow up of the French Polycythaemia Study Group study, rates of progression to MF and AML were 32% and 24% at 20 years respectively in HU-treated patients. No consistent long-term

data is available on the use of IFN-alpha, while in the RESPONSE trial at 80-weeks, a trend towards higher rates of transformation to PPV-MF and AML was observed in the ruxolitinib arm. Avoiding disease progression remains an unmet clinical need.

Is outcome improvement in low-risk patients needed and possible? With the standardization of cytoreductive therapy in the high-risk category, the rate of thrombosis has been significantly reduced from 4.01 to 2.93 per 100 patient-year. However, the rate of vascular events has remained stable over the years in the "low-risk" category (2.03 vs. 2.24). Accordingly, young patients with "masked-PV" showed a very high rate of thrombosis compared with overt PV (3.01 vs. 1.99 per 100 patient-years, respectively). These data suggest that current therapy for low-risk patients may be improved. With this aim, the Italian multicentre "low-PV" study (NCT03003325) is currently investigating whether the use of peg-prolin IFN-alpha-2b may improve patients' outcome compared to standard therapy.

Personalized medicine in MF: MF is a rare blood cancer with an incidence of 0.1 -1.0 per 100 000 persons per year. MF may occur as primary disease (PMF) or as evolution from ET and PV (PET/PPV-MF) and generally affects the elderly, with a median age of 70 at diagnosis. MF is characterized by clonal myeloproliferation with reactive bone marrow fibrosis, extramedullary hematopoiesis and abnormal cytokine expression. Clinical manifestations include anemia, hepato-splenomegaly, constitutional symptoms (e.g., weight loss, night sweats, fever), bone pain, pruritus, thrombosis and bleeding. According to the International prognostic Score System (IPSS), survival in MF ranges from 11.2 years in low-risk patients to 2.2 in high-risk. Main causes of death are leukemic transformation that occurs in 8% to 23% of patients in the first 10 years of diagnosis, cardiovascular events, and infections. Risk factors for leukemia-free survival include ≥3% circulating blasts, platelet count <100×10^9/L and presence of unfavorable karyotype(7). The clinical phenotype of MF is very variable among patients. Therefore, it is not possible to indicate a unique front-line therapy. Conversely, all therapeutic options need to be adapted to each individual patient, according to clinical needs, prognosis, goals of treatment and global health status (**Figure 2**).

Problem-oriented therapy: Since there is no curative therapy of MF besides allogeneic stem cell transplant (alloSCT), treatment is generally guided by the predominant clinical needs: anemia, splenomegaly and systemic symptoms.

Anemia: Most MF patients present or acquire anemia during the course of the disease, mainly due to splenic sequestration, stem cell hypoplasia, and bleedings. Specific treatment for anemia should be considered in case of hemoglobin <10g/dl. Anemia is generally treated with androgens (oxymetholone) and danazol, with an expected response rate of approximately 35%. Response to erythropoiesis stimulating agents (ESA) ranges 30-45%, but their use may promote spleen enlargement and leukemic transformation. Therefore, ESA may be considered in selected patients without splenomegaly and low EPO levels (below 500 UI). Immunomodulatory drugs include thalidomide and second-generation lenalidomide and pomalidomide. Tolerability is not excellent due to peripheral neuropathy/thrombosis (thalidomide), and myelosuppression (lenalidomide). A recent randomized study of pomalidomide *vs* placebo in MF patients with transfusion-dependent anemia did not show a significant advantage in the pomalidomide arm. Corticosteroids alone may also be used for the management of refractory anemia, with transient responses.

Splenomegaly and systemic symptoms: Splenomegaly and systemic symptoms are present in over 80% of MF patients at diagnosis and progress over time. Before the advent of ruxolitinib, HU and corticosteroids were the first-line therapy for symptomatic splenomegaly and symptoms, respectively, with partial and transitory clinical benefit. Their use is now largely superseded by JAK inhibitors. Ruxolitinib showed superiority over placebo and standard therapy in patients with intermediate-2– and high-risk MF in the 2 pivotal phase 3 studies, COMFORT-I and COMFORT-II. In these studies, ruxolitinib led to rapid and durable reductions in splenomegaly and symptom burden, with improvements in quality of life. The most common "on-target" adverse events were anemia and thrombocytopenia; also opportunistic and severe ruxolitinib-related infections require careful screening and monitoring. More recent post-hoc studies have indicated that ruxolitinib is also associated with a survival benefit compared to placebo or standard therapy, possibly due to reversal of cachexia and reduction of proinflammatory cytokines, known for their negative impact on survival(8). Splenectomy is indicated in patients with large and painful splenomegaly where JAK-inhibitors are not available or prove ineffective. It is burdened by a perioperative mortality rate of 5%–10% and by a morbidity rate up to 25%.

Risk-adapted therapy: Prognosis is a key concept when establishing a personalized treatment strategy. Operationally, MF patients are divided into two larger categories: higher risk, including high/intermediate-2 risk patients, whose median survival is lower than 5 years, and lower risk category, projected to a longer survival. The only treatment modality that may potentially cure MF is alloSCT, that is currently associated with at least 50% rate of transplant-related deaths or severe morbidity. The feasibility of alloSCT should be evaluated in all patients aged <70 years at intermediate-2 or high risk and in specific subset of lower-risk patients. The role of alloSCT in MF is addressed by Lussana *et al* in this issue.

Intermediate-2/high risk patients not candidates to alloSCT: Non-transplant candidates with survival probability inferior to 5 years at diagnosis should receive therapy that may target their main clinical needs. In case of symptomatic splenomegaly and/or symptoms, ruxolitinib may be considered the first therapeutic option. Hydroxyurea may also be used front-line in case of mild splenomegaly, leukocytosis and thrombocytosis, while ruxolitinib is indicated in case of severe splenomegaly and/or systemic symptoms. Patients should also be considered for clinical trials. Several preclinical combination treatments are currently being explored in order to increase efficacy and reduce toxicity. Among other JAK-inhibitors under clinical investigation, Pacritinib is the most promising. In the PERSIST trials, pacritinib showed superiority over BAT including ruxolitinib in terms of spleen and symptoms responses. Momelotinib is a JAK1/2 inhibitor with the unique characteristic to ameliorate anemia; results of 2 phase III studies (SIMPLIFY-1 & -2) are expected. Regarding non-JAK inhibitors, Imetelstat, a telomerase inhibitor, was found to induce molecular and marrow responses; the IMBARK study is now ongoing. PRM-151, a recombinant human pentraxin-2 is also under investigation in a phase 2 study (PROMOTE). Sotatercept and the SMAC mimetic (LCL161) are also being assessed in early phase studies.

Figure 2. Personalized medicine in MF. Risk stratification is based on the IPSS score at diagnosis and on the DIPSS during follow-up in PMF patients. Use MYSEC-PM in PPV/PET-MF patients.

Intermediate-1/low risk patients: Watchful waiting is a good option in asymptomatic patients with low/intermediate-1 risk disease. Cytoreductive therapy might be needed in the presence of extreme leukocytosis or thrombocytosis to reduce the thrombotic risk. Notably, current risk models for survival do not capture disease burden, and some of lower-risk patients may present significant splenomegaly, systemic symptoms and/or anemia requiring symptoms-adapted treatment including ruxolitinib. In a subgroup analysis of 163 MF patients at intermediate-1 IPSS risk treated with ruxolitinib in the JUMP trial (NCT01493414), efficacy and safety data were comparable to those observed in the total population. These data were confirmed by the UK Robust trial results, including 14 intermediate-1 risk patients. In a retrospective analysis on 408 MF patients treated with ruxolitinib according to prescribing obligations in 18 Italian Hematology Centers, intermediate-1 risk category and lower burden of the disease (e.g.: spleen palpable less than 10cm below costal margin, transfusion-independency, platelet count >200x10^9/L) significantly correlated with im-

proved probability of spleen response. A sub-analysis of 70 intermediate-1 risk patients, 54.7% and 80% of evaluable patients achieved a spleen and symptoms response at 6 months, respectively. These results, although derived from uncontrolled and non-randomized studies, suggest that symptomatic intermediate-1 risk patients may have significant clinical benefits from ruxolitinib therapy. Accordingly, the European label indicates ruxolitinib for patients with MF-related splenomegaly and/or symptoms, without favoring a risk-adapted treatment model as in the US indications(9).

Unmet clinical needs & unanswered questions. Should patients with PPV/PET-MF receive a personalized approach? Secondary MF has been generally equated to primary MF in terms of prognostic and therapeutic considerations. However, an increasing burden of evidence shows that PPV/PET-MF should be considered as a distinct entity. In a recent multicenter study involving 685 secondary MF, the *JAK2*^{V617F} mutation was associated with larger splenomegaly and shorter leukemia-free survival compared to CALR-mutated patients, that were projected to longer survival independently from the type of CALR mutations. Based on this cohort, the Myelofibrosis Secondary to PV and ET-Prognostic Model (MYSEC-PM) was developed considering: hemoglobin <11g/dl, circulating blasts ≥3%, CALR-unmutated genotype, platelet count <150×10^9/l, constitutional symptoms, and age. Patients were allocated into four risk categories with different survival (P<0.0001). In the near future, the MYSEC-PM should enter clinical practice and drive therapeutic decision-making in these patients(10).

How (if so) should patients at intermediate-1 risk be selected for ruxolitinib therapy? Data regarding ruxolitinib efficacy and safety in the intermediate-1 risk category derive from uncontrolled and sometimes retrospective studies. Lacking evidence of a comparative advantage of ruxolitinib over standard treatments in this population, the use of ruxolitinib should be reserved to patients with severe impairment in quality of life due to severe and/or symptomatic splenomegaly and/or severe systemic symptoms. Splenomegaly may be defined severe if palpable 15 cm below the costal margin; however, this threshold should be adapted case-by-case. The MPN-10 total symptoms score represents an easy-to-assess and well standardized tool to quantify symptoms in MF. Its assessment is necessary prior to treatment decision and to verify responses. The ELN-SIE recommendations recently indicated an MPN-10 threshold of 44 to identify patients with severe symptoms.

Future perspectives: Despite a great improvement in the knowledge of biological, prognostic and therapeutic aspects in PV and MF, several issues still need to be addressed. Strategies to reduce the risk of myelofibrotic/leukemic progression are lacking, together with a medical approach with disease-modifying potential. Particularly, type II JAK inhibitors, mutant-specific JAK2 inhibitors, and mutant CALR immunotherapy are being explored to improve clonal selectivity. Prognosis and the role of targeted therapies in early-PMF still need to be investigated in prospective cohorts; additionally, it is still unclear whether an early treatment could improve long-term outcome.

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TREATMENT OF LIGHT CHAIN AMYLOIDOSIS

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With an estimated incidence of 1 case per million person year, immunoglobulin light chain (AL) amyloidosis is the most common form of systemic amyloidosis, accounting for 10-12% of patients who progress from monoclonal gammopathy of undetermined significance (MGUS). This disease is caused by misfolding, aggregation and deposition in tissues of light chains produced from usually small plasma cell clones that infiltrate the bone marrow by <10% in 50% of cases. The process leading to the formation of amyloid deposits in target organs causes progressive organ damage eventually leading to organ failure and death if it is not recognized at an early stage and effectively treated (1). Despite recent advances in the management of this disease, still approximately 30% of patients die within the first year from diagnosis, because they are diagnosed only after irreversible cardiac damage has ensued (1). Symptoms of organ involvement are late and mimic more common diseases, and 40% of subjects are diagnosed more than one year after the disease has become symptomatic. However, organ involvement by AL amyloidosis can be detected at a pre-symptomatic stage with biomarkers, N-terminal pro-natriuretic peptide type-B (NT-proBNP) for the heart, albuminuria for the kidney, and alkaline phosphatase for the liver. Moreover, increased circulating free light chains (FLC) consistently precede the presentation of AL amyloidosis by 4 years or more. Thus, we advocated screening with biomarkers of amyloid organ involvement of all patients with MGUS and abnormal FLC ratio. Although some clinical features, such as the association of a monoclonal component, heart or soft tissue involvement, and albuminuria, can in some instances strongly suggest AL amyloidosis, there is often substantial overlap in the clinical presentation of different types of systemic amyloidosis. Thus, unequivocal typing of the amyloid deposits is mandatory before starting specific treatment. Immune fluorescence and standard light-microscopy immunohistochemistry have poor specificity, and reliable techniques, such as immunohistochemistry with custom-made antibodies, immuno-electron microscopy, or mass spectrometry, should be employed referring patients to specialized centers (1). DNA analysis is required to confirm hereditary forms. The therapeutic approaches targeting the amyloidogenic plasma cell are usually borrowed from multiple myeloma. However, patients with AL amyloidosis not only have a hematologic malignancy, but their multi-organ involvement makes them particularly fragile and susceptible to treatment toxicity. Thus, the treatment of patients with AL amyloidosis needs a critical level of expertise, and should be risk-adapted, carefully balancing treatment efficacy and toxicity, with close, specialized monitoring of fluid retention, hypotension, and cardiac and renal function. A close monitoring of hematologic and organ response to treatment with validated biomarker-based response criteria is also mandatory (2). The treatment plan should be reconsidered every two or three cycles or three months after autologous stem cell transplantation (ASCT), being ready to shift patients to rescue therapy based on the quality of response (1). To date, no randomized clinical trials of modern treatment approaches have been published. Thus, whenever possible, patients should be referred to specialized centers for treatment and inclusion in clinical trials. Low-risk patients represent approximately 15% of all subjects with AL amyloidosis and are candidates for ASCT (1). This procedure is associated with a substantially higher risk of early mortality in AL amyloidosis compared to multiple myeloma. The great majority of transplant related deaths occur in patients with elevated cardiac biomarkers, and subjects whose NT-proBNP is >5000 ng/L and/or cardiac troponin T is >0.06 ng/mL should not be offeres ASCT. Other eligibility criteria for ASCT are age <65 years, performance status (Eastern Cooperative Oncology Group) 0-2, eGFR >50 mL/min per 1.73 m^2 unless on dialysis, New York Heart Association (NYHA) class <III, cardiac ejection fraction >45%, systolic blood pressure >90 mmHg (standing), and lung CO diffusion capacity >50% (1). Accumulation of a critical level of experience in transplanting patients with this disease is also crucial,

the outcome being significantly poorer at centers where less than 4 transplants per year are performed in patients with AL amyloidois (3). When adequate selection of transplant candidates is applied at referral centers the outcome of patients with AL amyloidosis undergoing ASCT is excellent, with hematologic response in approximately 70% of subjects and complete response (CR) in 35-37%. Patients who fail to reach CR after ASCT can receive adjuvant bortezomib-based treatment, increasing the CR rate to almost 60%. Bortezomib can be used as induction therapy before ASCT, and this approach increases the response rate and quality of response, particularly in patients with a bone marrow plasma cell infiltrate >10%. Intermediate risk patients account for approximately 65% of patients with AL amyloidosis (1). Until recently, a standard treatment for these patients has been oral melphalan / dexamethasone (MDex). This regimen is very well tolerated and yields a 76% overall hematologic response rate, with CR in 31% of cases. The availability of the proteasome inhibitor bortezomib was enthusiastically welcome, because the amyloidogenic plasma cell use the proteasome to cope with the toxicity generated by the toxic plasma cell they produce. Indeed, we have recently shown that amyloidogenic light chains are intrinsic stressors for plasma cells and increase their sensitivity to proteasome inhibition. In the largest study of frontline treatment with cyclophosphamide / bortezomib / dexamethasone (CyBorD) of patients with AL amyloidosis, the overall hematologic response rate was 60%, with CR in 23% of cases (4). An international phase III study (NCT01277016) comparing MDex with bortezomib plus MDex (BMDex) has recently been completed, showing a significantly higher overall hematologic response rate with BMDex (81% vs. 57%, P=0.005), with longer time to second-line therapy or death and comparable rates of CR (23% vs. 20%). Based on this data, bortezomib should be offered to intermediate-risk patients, in the absence of contraindications such as peripheral neuropathy. The choice of the best combination should take into account clonal and patient characteristics. Treatment with BMDex should be considered a new standard of care and has the advantage of "universally" overcoming the effects of both gain 1q21 (poor outcome with oral melphalan) and t(11;14) (poor outcome with bortezomib) (5). Treatment with CyBorD or bortezomib / dexamethasone alone is preferred in patients with potentially reversible contraindications to ASCT, being stem cell sparing (1). The remaining 20% of patients with renal AL amyloidosis are high-risk, most frequently because of advanced cardiac stage (stage IIIb, NT-proBNP >8500 ng/L and troponin I >0.1 ng/mL) (1). High-risk patients are treated with low-dose combinations, with weekly dose escalation based on tolerability, under intensive monitoring (1). So far, no treatment approach, was able to overcome the poor prognosis of these patients, whose median survival ranges from 3 to 7 months. Nevertheless, the few stage IIIb patients who survive long enough (at least 3 months) to take advantage of response to chemotherapy have a significantly better outcome (4). Upfront therapy can be repeated at relapse, if possible, although this is associated with shorter time to retreatment without reduction in overall survival. Lenalidomide and pomalidomide can be used in refractory patients, being able to overcome resistance to alkylating agents, proteasome inhibitors, and thalidomide. However, the use of lenalidomide is associated with worsening renal failure in patients with nephrotic syndrome. Hematologic response to pomalidomide is rapid (median 1 months) and is observed in up to two thirds of patients in the refractory setting (6). Newer agents have proven effective in relapsed/refractory patients, and are being considered for novel upfront combinations. The oral proteasome inhibitor ixazomib was particularly active in bortezomib-naïve patients, and is currently being tested in a randomized phase III trial (NCT01659658) (7). Two trials of this agent in combination with cyclophosphamide and dexamethasone (NCT03236792, NCT01864018) are ongoing in the upfront setting. Daratumumab is a very promising drug in AL amyloidosis. A recently published series showed a 76% hematologic response rate with 36% CRs in a median time of 1 month (8). Two phase II trials of daratumumab in relapsed / refractory patients are underway (NCT02841033, NCT02816476) and a phase III randomized trial of daratumumab in combination with CyBorD vs. CyBorD alone in newly-diagnosed patients is about to begin (NCT03201965). New therapeutic approaches specifically targeting the amyloid deposits or interfering with amyloid formation and organ targeting are emerging as a possible complement of chemotherapy. For some of them early clinical data have been published. Our observation that the anthracycline 4'-iodo-4'-deoxy-doxorubicin inhibited amyloidogenesis *in vitro* and could induce clinical improvement in patients with AL amyloidosis, prompted the investigation of related non-cytotoxic compounds. Amongst them, the antibiotic doxycycline proved able to disrupt the amyloid fibrils in transgenic mouse models of transthyretin and AL amyloidosis. Moreover, doxycycline can interfere with light chain-induced toxicity in a C. elegans model, in which exposure to amyloidogenic light chains from patients with cardiac AL amyloidosis results in the reduction of the pumping function of the nematode's pharynx which resembles the vertebrates heart. A recent retrospective case-control study showed that patients with cardiac AL amyloidosis who received doxycycline during chemotherapy had a reduction in early mortality, translating into higher response rates and prolonged survival (9). A prospective, international, randomized trial of chemotherapy with or without doxycycline is being designed. The London group developed a palindromic compound, CPHPC, that is a competitive inhibitor of the binding of serum amyloid P component (SAP) to amyloid fibrils, which protects them from degradation, and is able to remove SAP from the bloodstream. Subsequently, they showed that administration of anti-human-SAP antibodies to mice with amyloid deposits containing human SAP triggers a complement-dependent, macrophage-derived giant cell reaction that removes visceral amyloid deposits, and proposed a combination approach based on CPHPC and anti-SAP antibodies. A pilot clinical study of this approach showed encouraging results, and a clinical trial in patients with AL amyloidosis who undergo or have completed chemotherapy is ongoing (NCT03044353). Future trials based on validated organ response criteria are eagerly awaited. Currently, the immunotherapy targeting amyloid deposits in the most advanced stage of clinical development is based on NEOD001, a monoclonal antibody that binds to amyloid protofilaments and fibrils. In a phase I/II study on patients with AL amyloidosis who had completed chemotherapy and had persisting organ dysfunction, cardiac and renal response rates were 57% and 60%, respectively (10). The results of this study were recently updated, showing that organ response to NEOD001 was independent of rate and depth of previous hematologic response. Two phase III randomized, placebo-controlled trials of NEOD001 combined with bortezomib-based chemotherapy in newly-diagnosed patients (NCT02312206), and as single agent in subjects who completed chemotherapy (NCT02632786) have recently completed enrollment and results are eagerly awaited. Chemotherapy reducing the supply of the toxic light chain is the backbone of treatment of AL amyloidosis. Regimens increasing the rate and depth of hematologic response significantly improved the outcome of patients with this diseases in the last few years. The availability of newer, even more powerful anti-plasma cell agents, such as pomalidomide and daratumumab, that will soon be tested in clinical trials in combination with established regimens, will most likely further improve the outlook of patients with AL amyloidosis. However, many patients are still diagnosed when advanced organ damage has already established, and early diagnosis, at a pre-symptomatic stage, based on screening of patients at risk with sensitive biomarkers remains critical to improve survival in this disease. Several strategies are being developed to interfere with the amyloidogenic process and target the amyloid deposits in combination with chemotherapy or after hematologic response has been reached. If this approach succeeds, in the near future anti-plasma cell and anti-amyloid drugs will be combined, potentially further improving survival and quality of life of patients with AL amyloidosis, moving towards a cure for this dreadful disease.

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IMMUNOTHERAPY: MONOCLONAL ANTIBODIES AND T CELL ADOPTIVE THERAPY

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Multiple Myeloma (MM) is a malignancy associated with significant immune dysfunction imparted both by the disease itself as well as by many of the immunosuppressive therapies that have been used in the past. Although the use of new agents, both in the frontline and in relapsed setting, have resulted in significant improvements in patient outcomes, drug resistance remained an inevitable challenge. Immunotherapy is a promising field in cancer research and includes both non-cellular immunotherapy and cell-based immunotherapy. Over the past decade, therapy based on monoclonal antibodies (MoAbs) has demonstrated efficacy against several B-cell malignancies. For example, the anti-CD20 MoAb rituximab is indicated for the treatment of non-Hodgkin's lymphoma and B-cell chronic lymphocytic leukemia [1]. The success of MoAb-based therapy in these and other cancers has led to the investigation of MoAbs in the treatment of MM. MoAbs may induce cytotoxic action directly, by blocking activation signals necessary for cells growth or viability, interfering with the interaction between the ligand and its receptor or inducing modulation of the receptor. Alternatively, they may exert their effects through antibody-dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). The ADCC mechanism depends on the activation of FC-receptors on myeloid and NK cells by tumor cell attached immunoglobulins. Subsequent cytoxicity is achieved through perforin and granzymes release or by involving death ligands and TNF-related apoptosis-inducing ligand. The CDC process is mediated by interaction between antibody Fc domains and complement activating proteins that leads to induction of cellular membrane attack and subsequent cell death. The activation of ADCC/CDC mechanisms can also stimulate T-cell immunity through the production of cytokines, chemokines and opsonins[2]. Different MoAbs targeting adhesion molecules showed promising results both in pre-clinical and in clinical studies and, some of them, such as elotuzumab (ant-CS1) or daratumumab (Anti-CD38), are approved also in Europe. **Elotuzumab** is a humanized immunoglobulin G1 MoAb directed against the cell surface glycoprotein CS1 (CD2 subset 1), that binds with high affinity to MM cells and blocks their adhesion to bone marrow stromal cells, which potentially overcomes the stimulatory effects of bone marrow stromal cells on MM growth and survival[3]. The primary mechanism of action of elotuzumab is NK cell–mediated antibody-dependent cellular cytotoxicity (ADCC). In phase I/II studies, elotuzumab as monotherapy, in relapsed/refractory MM, demonstrated 32% stable disease and encouraging clinical activity (81% to 82% objective response [OR]) when combined with lenalidomide and dexamethasone, suggesting synergy.[4] In the phase 3 trial, called ELOQUENT-2[5], in patients with relapsed or refractory multiple myeloma, the efficacy and safety of elotuzumab in combination with lenalidomide and dexamethasone was evaluated comparing their results with those obtained using lenalidomide and dexamethasone without elotuzumab. After a median follow-up of 24.5 months, the overall response rate in the elotuzumab group was 79%, *versus* 66% in the control group (P<0.001). The rate of progression-free survival at 1 year in the elotuzumab group was 68%, as compared with 57% in the control group; at 2 years, the rates were 41% and 27%, respectively. Median progression-free survival in the elotuzumab group was 19.4 months, *versus* 14.9 months in the control group (hazard ratio for progression or death in the elotuzumab group, 0.70; 95% confidence interval, 0.57 to 0.85; P<0.001). Common grade 3 or 4 adverse events in the two groups were lymphocytopenia, neutropenia, fatigue, and pneumonia. Infusion reactions occurred in 33 patients (10%) in the elotuzumab group and were grade 1 or 2 in 29 patients. **Daratumumab** is a human IgG1 antibody that targets CD38, a 46-kD type II transmembrane glycoprotein that is abundantly expressed on malignant plasma cells. This antibody has shown impressive single-agent activity in a very heavily pretreated patient population and was approved in the US by the Food and Drug Administration (FDA) and in Europe by the European Medicines Agency (EMA) for patients who had received three prior lines of therapy including an immunomodulatory agent and a proteasome inhibitor. Evidence for the efficacy and safety of daratumumab, 16 mg/kg, monotherapy is available from the pivotal Phase II trial MMY2002[6], which included patients with MM who are heavily pretreated and highly refractory. Even in this severe patient population, daratumumab provided compelling efficacy with an overall response rate (ORR) of 29%. This response rate was supported by results from the Phase I/II study GEN501[7], which showed an ORR of 36%, with a very good partial response (VGPR) or better of 12% (including 3 stringent complete responses [sCR]). Responses to daratumumab monotherapy were rapid and durable: the median time to response was 1 month and the median duration of response was 7.4 months. In the pivotal Study MMY2002 the median PFS was 3.7 months and the median OS 17.5 months after a median follow-up of 14.7 months. Median OS has not yet been reached in the supportive Study GEN501, with 74% of subjects still alive after a median follow-up of 15.2 months and the pooled analysis of data from MMY2002 and GEN501 demonstrates a median OS of 19.9 months after a median follow-up of 14.8 months. Daratumumab provides compelling clinical activity in a broad range of MM subgroups, including patients who are refractory to pomalidomide and carfilzomib and is well tolerated, with a low frequency of clinically manageable adverse events and a low rate of treatment discontinuation. The main side effect of the antibody was infusion-related reactions (IRRs) that occurred in the majority (90%) of patient during the first infusion without treatment discontinuation. There are a number of ongoing phase II and III trials involving SLAMF7 and CD38 antibodies as a backbone of lenalidomide, pomalidomide and bortezomib. In the relapsed setting, elotuzumab is being added to a backbone of pomalidomide-based therapy and compared directly to nivolumab (an anti PD-1) in a large phase III trial. Concerning daratumumab, although it is effective as monotherapy, and is approved in either patient with double-refractory disease to a PI and IMiDs or in patients who have had at least 3 lines of prior therapy the overall response rate substantially improves when daratumumab is combined with lenalidomide and dexamethasone or bortezomib and dexamethasone. The era of MoAb therapy in MM is just beginning, a number of others MoAb are in various stages of clinical development, including 2 more MoAb anti CD38, the SAR 650984 and MOR202, that are under investigation in phase I studies, and those targeting myeloma cell surface antigens, the bone marrow microenvironment, and immune effector T cells such as anti-programmed cell death protein 1 antibodies. Immune-check point inhibitors targeting the PD-1/PD-L1 axis have recently emerged as promising agents that control antitumor immune responses. Increased expression of PD-L1 in malignant plasma cells and upregulation of PD-1 on effector T and NK cells of MM patients provided a rationale that targeting PD-1 may be an effective therapeutic approach in MM[8]. However, no objective response was obtained using the anti PD1 MoAb alone and the observation that IMiDs increase effector immune cell activity provided rational for phase I study using the anti PD1 MoAb in combination with lenalidomide or pomalidomide that are now under evaluation. In addition, cellular immunotherapy using dendritic cell (DC) vaccination and adoptive immunotherapy with chimeric antigen receptor (CAR) T cells or T cell receptor (TCR)-engineered T cells are emerging as promising treatment strategies for MM. **Chimeric antigen receptor T cells (CARs)** are engineered molecules that fuse the specificity of a monoclonal antibody with the activation of the T-cell receptor signaling domain. It is a new method for tumor adoptive immunotherapy. CAR T cells are genetically modified to express an antigen receptor recognizing a tumor associated surface antigen. Ideally, these tumor associated antigens would be uniquely expressed by the malignant cells and absent on normal tissues. Only a limited number of MM patients have received CAR T cell therapy but preliminary results have been encouraging[9]. Advances are being made using CAR T cell technology to target myeloma antigens such as B cell maturation antigen (BCMA), CD138, and kappa-light chain as well as CD19 on putative myeloma stem cells. CARs have the potential for offering definitive therapy for MM. Overall the im-

munotherapy are likely to become part of therapy available for MM patients.

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DIFFUSE LARGE B-CELL LYMPHOMA: THE RELEVANCE OF GENE EXPRESSION PROFILING

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Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease in adult patients with respect to clinical presentation, histopathology and molecular-genetic features. While the long-term cure rate after rituximab-containing conventional chemotherapy programs exceeds 80% in young patients with low and low-intermediate risk according to the International Prognostic Index (IPI), similar treatments remain unsatisfactory in patients belonging to high and high-intermediate risk groups.[1] For these patients, the optimization of front-line therapy remains an important objective. Advances in molecular genetics have significantly improved the understanding of the biological diversity in DLBCL, leading to the discovery of key oncogenic pathways and novel therapeutic targets. In 2000, the NCI group first showed that DLBCLs could be divided in at least two main categories based on their gene signature related to germinal centre B-cells (GCB) and activated lymphocytes from the peripheral blood (ABC), respectively.[2] Such distinction, which could not be made by histology, was provided with important prognostic implications, the ABC tumours showing a significantly worse outcome and resistance to conventional anthracycline-based therapies. A few years later, a study held by the Lymphoma Leukemia Molecular Profiling Project (LLMPP) and based on some hundred DLBCLs confirmed the original observation of the NCI Group by using a chip measuring the expression of all encoding genes.[3] In this study, a third category was reported in between the GCB and ABC ones. Importantly, the subclassification of DLBCLs based on gene expression profiling (GEP) has maintained its value also in the present immuno-chemotherapy era.[4] However, all the above-mentioned studies had been carried out on m-RNA extracted from fresh or frozen samples, available in only a few patients. Therefore, several immunohistochemical algorithms were developed to surrogate the results of GEP on formalin-fixed, paraffin-embedded (FFPE) tissue samples.[5,6] However, none of them produced the expected results in terms of both inter-lab reproducibility and prognostic value.[7,8] They do not distinguish DLBCLs into GCB and ABC but else in GCB and non-GCB, the latter representing a kind of Pandora's box. In 2014, the LLMPP proposed a new approach applicable to mRNA extracted from FFPE biopsies.[9] It was based on a panel of 20 genes (15 top-genes and 5 housekeeping genes) and the usage of the NanoString platform, which requires neither amplification nor retro-transcription of m-RNA. The approach was applied to 67 archival DLBCLs, which had been treated by R-CHOP. It performed as well as conventional GEP and much better than three different immunohistochemical algorithms. The approach did also allow the identification of the third group between the GCB and ABC ones: it was termed "Unclassified" but behaved closer to the GCB cases.

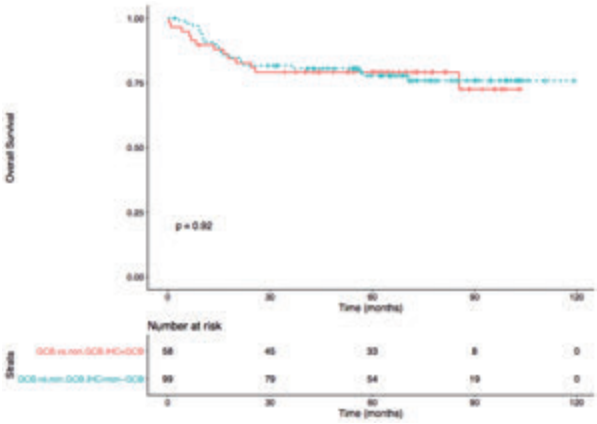

Figure 1A: Immunohistochemistry does not predict the outcome of the patients with DLBCL. The GCB and non-GCB groups show an identical behaviour.

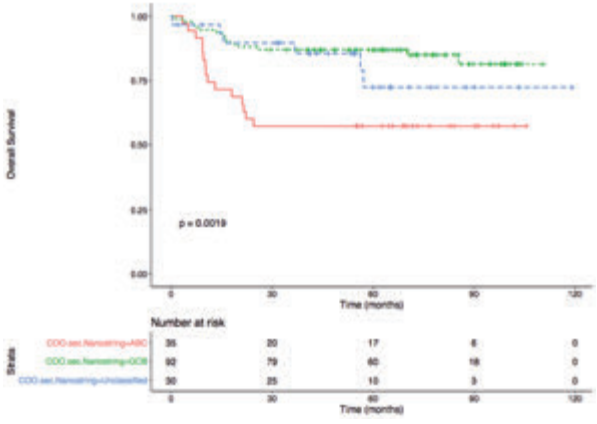

Figure 1B: The determination of the cell of origin based on the NanoString approach shows that GCB and ABC DLBCLs have a significantly different response to therapy and clinical outcome.

The relevance of this molecular classifier has so far been confirmed by a few studies.[10] Recently, the German Group did not find a significant prognostic difference between GCB and ABC DLBCLs assessed by the LLMPP approach, while confirming the lack of correspondence between molecular and immunohistochemical data.[11] However, the results of this study were obtained either in young patients receiving etoposide-containing chemotherapy regimens (R-CHOEP14) or elderly with possible co-mobilities undergoing R-CHOP14. In the recently published Italian phase III randomized trials DLCL04 and RHDS0305, 645 untreated young (less than 65 years-old) DLBCL patients with a high-intermediate or high IPI score were enrolled.[12,13] The aim of DLCL04 was to assess the possible benefit of intensification with high-dose chemotherapy and autologous stem-cell transplantation and two different doses of rituximab and doxorubicin-based chemoimmunotherapy (R-CHOP 14 and R-MegaCHOP14) compared to chemoimmunotherapy alone, while the RHDS0305 intended to test the efficacy of R-CHOP14 vs. R-HDS, which includes ASCT front-line.

In both studies, the definition of the cell of origin (COO) by immunohistochemistry was once again unable to discriminate the clinical outcome according to the allocated treatment.[12,13] Finally, yet importantly the Revised WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues has established that the determination of the COO becomes mandatory for DLBCLs of the NOS type.[14] Although immunohistochemical algorithms are quoted as acceptable, the molecular determination of the COO is indeed preferred as underlined by the inclusion of a dendrogram taken from the paper of Scott *et al.*[9] We performed digital multiplex gene expression profiling (NanoString technology) in 214 of the 645 patients enrolled in the two above mentioned Italian trials, for whom FFPE material had been centralised. One-hundred and fifty-seven cases corresponded to DLBCLs of the NOS type: to them we will refer in the following. By applying a 28 gene-panel, the cases were molecularly subdivided into GCB, ABC or Unclassified. The panel contained the 20 genes used to assign the COO subtype according to the LLMPP algorithm.[9] The remaining 8 genes were employed to assess MYC and BCL2 expression, to quantify TP53 level, to detect potential therapeutic targets (STAT3, NFKB, PTEN, PKI3CA), and to explore the expression of CD68 as a microenvironmental clue. We confirmed that the distinction of DLBCLs into gene expression-based COO categories is more accurate than immunohistochemistry (IHC). In particular, 58 and 99 cases were respectively classified as GCB and non-GCB, by the Hans immunohistochemical algorithm.[5] Conversely, the NanoString approach subdivided the same cases into 92 GCB, 35 ABC and 30 Unclassified tumours. These findings highlight that the immunohistochemical non-GCB category was composed for 39.4, 32.3 and 28.3% of molecular GCB, ABC and Unclassified cases. Indeed, considering the entire cohort, the Overall Survival (OS) was significantly different in GCB compared to ABC patients, with the Unclassified group being quite close to the GCB one, while IHC failed to identify prognostic subgroups (Figures 1A and 1B). In the RHDS0305 trial, no significant difference of OS was observed between the two therapeutic arms (R-CHOP14 vs. R-HDS), when the analysis was performed on all the profiled patients (62) or the GCB ones only (37). Instead, in the ABC group (10 patients) a clear difference was recorded between R-HDS and R-CHOP14 with 83% and 25% of patients in stable complete remission (CR) after 9 and 5 years, respectively. Because of the practical implications of this finding, further cases enrolled in the RHDS0305 trial are under investigation at present to verify the possible higher efficacy of R-HDS in ABC patients. Furthermore, we investigated the possible correlation between the immunohistochemical determination of MYC and BCL2 proteins and the respective mRNA expression data assessed by NanoString. No linear relationship was seen between the expression of MYC and BCL2 mRNA and corresponding proteins, although all the cases negative on IHC turned out to be down-regulated on gene expression. Moreover, BCL2 and MYC mRNA expression showed a significantly higher predictive value in terms of OS than IHC, when evaluated singly or in combination. Finally, since the status of *BCL2*, *MYC* and *BCL6* assessed by FISH was available in all the profiled cases, it was checked whether double or triple hits had any relationship with the molecular groups. It was observed that most if not all of them occurred among GCB cases. Thus, the assessment of the COO may contribute to the screening of DLBCL patients, to whom FISH studies should be usefully applied. Our results suggest that molecular definition of the COO of DLBCLs is crucial to identify patients, who are at high risk of poor outcome, when treated with R-CHOP, and may benefit by intensified high dose chemotherapy programs or experimental new treatments.

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NEW MECHANISMS OF IRON TOXICITY

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Abstract: Iron is an essential element for the normal cellular life but when it exceeds the needs of physiological cellular processes, reactive oxygen species (ROS) are produced. Increased ROS levels may have healthful or deleterious effects depending on their levels. In the case of imbalance in redox homeostasis, ROS levels overwhelm cellular antioxidant defenses, and oxidative stress is established. Many biological functions are dependent upon appropriate intracellular ROS levels, and are deregulated in cells under oxidative stress conditions. These processes involve the activation of signaling pathways leading to alterations of cellular cycle, proliferation, differentiation, and eventually cell death. ROS are also considered crucial players in the initiation and progression of hematological malignancies such as Myelodysplastic syndrome (MDS). In MDS iron overload, mainly from dyserithropoiesis and transfusion dependency, lead to an imbalance of redox homeostasis and therefore to free iron toxicity. The aim of this paper is to summarize the main evidences on new mechanisms of iron toxicity in order to improve our tools to predict outcome and eventually to individuate new targeted therapy.

Itroduction: The imbalance between oxidants and antioxidants leads to a cellular state known as oxidative stress. Excessive cellular ROS production and/or deficient antioxidant defenses have been found in several diseases, including hematopoietic malignancies. At low/moderate levels ROS have beneficial effects activating cellular pathways involved in physiological responses; however, at high concentrations, they induce oxidative stress and consequently adverse modifications in DNA, protein and lipids. These macromolecules damage lead to changes in gene transcription and deregulation of signaling pathways, as well as to chromosome instability, genetic mutation and epigenetic abnormalities (1). MDS is a type of hematological malignancies characterized by cytopenias, dysplasia, increased apoptosis, and ineffective hematopoiesis in one or more of the myeloid lineages. Patients with MDS require chronic red blood cell (RBC) transfusion due to anemia. Multiple RBC transfusions cause secondary iron overload and subsequent excessive generation of reactive oxygen species, through the chemical Fenton reaction. An altered ROS homeostasis lead to a persistent state of oxidative stress that through several mechanisms may affect bone marrow cells growth, including hematopoietic stem cells (HSCs), progenitors (HPCs) end microenvironment. The regulatory pathways that control HSCs and HPCs cell cycle, proliferation and differentiation are largely mediated by cytokines and growth factors, such as interleukins (Ils), stem cell factor (SCF), colony-stimulating factors (CSFs), interferons (IFNs), erythropoietin (EPO), and thrombopoietin

(TPO), as well as their receptors, such as granulocyte colony-stimulating factor receptor (GCSFR), erythropoietin receptor (EPOR), thrombopoietin receptor (TPOR or c-MPL). The "home" of hematopoietic cells, called niche, is characterized by a complex cross-talking between the above mentioned elements. The correct fate decisions constantly have to be chosen by HSCs, and the exact timing and sequential order of all choices in each cell support normal hematopoiesis. Every interferences in this process may change the result. In particular the HSCs fate are determine by their self-renewal and proliferation/differentiation capacity (2). Recent studies suggest that ROS plays a role in HSCs state and function and it has been well described how ROS levels are essential to maintain HSCs self-renewal capacity(3). Appropriate understanding of the complex relationship between hemopoietic niche function, iron and oxidative stress become essential to add diagnostic, prognostic and possibly therapeutic tools in hematologic malignancies as MDS.

Iron homeostasis in healthy and disease: From the biochemical point of view, iron has various oxidation states ranging from Fe^{2-} to Fe^{6+} ,with the Fe^{2+} (ferrous) and Fe^{3+} (ferric) states being the most biologically relevant. This flexibility makes iron suitable for a variety of normal biochemical reactions particularly involving electron transport and mitochondrion activities. To maintain a steady level of iron and a normal iron homeostasis, living organisms must be able to release stored iron during iron deficiency and store excess iron during iron sufficiency in an appropriate manner. Regulation of many of the "iron genes" depends on the presence of iron response/regulatory element (IRE) in the mRNA and Iron response/regulatory proteins (IRPs) 1 and 2. Under normal circumstances, systemic iron balance is primarily maintained by the iron regulatory hormone hepcidin that binding to ferroportin (FPN) on the cell surface regulates the iron cellular efflux. When iron overwhelm the body's capacity to store and transport iron, non-transferrin bound iron (NTBI) appears in the circulation and its sub component, labile plasma iron (LPI), enter into the cell through canonical but also alternative channels and participate to increase labile cell iron (LCI). Excess LCI can enter the mitochondria and become non-chelatable by high-affinity iron chelators in the cytosol. The LCI surplus take part to increase the ROS production. Usually the cell holds physiological adaptation mechanisms against the ROS level increases, mainly mediated by the activation of nuclear factor erythroid 2-related factor (Nrf2). Oxidative stress, normally product into the cell, halts Nrf2 degradation and allows it enter the nucleus and up-regulate the expression of antioxidant enzymes. Although Nrf2 can protect normal cells from oxidative stress when LCI increase this mechanism become inadequate to control the ROS surplus. A growing body of evidence describe how oxidative stress and iron overload can modulate several signaling pathways (such as Akt, p53 and Wnt,) which promote cell survival, avoid apoptosis, allow escape from growth arrest, and facilitate cancer transformation. In fact ROS contribute to carcinogenesis not only through genetic mutations but also through cell signaling deregulation. Actually ROS are involved in a complicated web of signaling networks where their generation is regulated by multiple pathways. Conversely, ROS act as signaling molecules for other signaling pathways such as PTEN, PTP1B, MAPK and NF- B involved in different way with the HSCs fate (4) Ludin and colleagues (3) showed *in vitro* how oxidative stress influences the fate of haematopoietic stem cells by compromising migration, development, self-renewal and cell cycle status. They showed how HSC in its quiescent state need to reside in an hypoxic, low ROS level, area of the niche. Extremely low ROS levels can cause defects in their differentiation ability leading to impaired repopulation capacity, on the other hand an adequate increases in ROS levels, drives stem cell differentiation to short term repopulating cells and further on to myeloid differentiation, moving the HSC to a more oxygenate area of the niche (the blood vessel compartment). Instead exceedingly high ROS levels, as may occur during important oxidative stress conditions can promote stem cell exhaustion and subsequent apoptosis. The emerging idea is that the ROS levels are crucial to settle the HSC development or dead. The obvious consequence of these *in vitro* evidences is that reduce oxidative stress may ameliorate all the clinical situation in which iron toxicity could be involved.

Oxidative stress and hematopoietic insufficiency: Several retrospective studies have suggested the beneficial effect of iron chelation therapy (ICT) to prolong survival in iron overloaded MDS patients. (5). Few iron overloaded MDS patients experience hematological improvements upon ICT (6). One of the possible explanation of the observed hematological improvement may be the consequence of reduced iron-related oxidative stress by ICT. In 2013, Chai and colleagues established an iron overloaded mouse model to investigate the effects of iron overload on haematopoietic stem and progenitor cells (HSPCs). Results show that iron overload markedly decreased the ratio of immature haematopoietic cells and reduced HSPCs clonogenic capacity. Iron overload increased ROS levels of HSPCs through the NOX4/ROS/P38 MAPK signalling pathway. (7). Similar results were found using bone marrow mesenchymal cells (BM-MSCs) in a similar murine model suggesting that iron can impair not only the HSPCs clonogenic capacity but similarly the quantity and quality of BM-MSCs and the bone marrow microenvironment also(8). The effect of oxidative stress on hemopoiesis has been investigated also in the transplant setting. A murine model was used to investigate the possible relationship between iron overload and engraftment post-allogeneic haematopoietic stem cell transplant (HSCT). Donor bone marrow mononuclear cells (BM-MNCs) from iron overloaded mice and normal mice were transplanted into recipient mice. Flow cytometry analysis of peripheral blood cells from the recipient mice, after transplantation, demonstrated that recipient mice of iron-overloaded donor, had lower levels of myeloid B and T-lymphocytic lineage engraftments compared to the recipient mice of normal donor. A different conclusion was described by Okabe and colleagues (8) which showed in an iron overloaded mouse recipient that oxidative stress could affect the engraftment of HSC from a normal donor by modifying microenvironment and remarkably reducing expression of CXCL12, VCAM-1, Kit-ligand, erythropoietin and thrombopoietin. They concluded that iron overload can damage bone marrow stromal and other key organs (liver, kidney) and therefore, indirectly, haematopoiesis. Interestingly, in almost the above murine models, hematopoietic insufficiency improved by treating mouse HSCs with iron chelator or the powerful antioxidant N-acetyl-cysteine (NAC), conveying that iron overload may be closely related to high oxidative stress (7).

Table 1. Summarizes the hematological processes in which iron overload and cellular oxidative stress are involved.

STEM CELL PROCESS	RESULTS
HEMATOPOIETIC INSUFFICIENCY	➢ iron overload markedly decreased the ratio of immature haematopoietic cells and reduced HSPCs clonogenic capacity ➢ iron can impair the quantity and quality of mesenchymal stem cells and the bone marrow microenvironment therefore, indirectly, haematopoiesis
CLONAL EVOLUTION	➢ oxidative stress may contribute to an AML-promoting effect ➢ increased ROS levels stimulate leukaemogenesis through the regulation of redox-sensitive transcription factors (Nrf2, Bach1, NF- kB, HIF1) or alterations of key pathways, including activation of Akt, NF-κB, Wnt, antioxidant defenses, and DNA damage response, as well as inactivation of JNK, C/EBPδ, and PTEN
DNA METHYLATION	➢ ROS might modulate the DNA methylation ➢ oxidative stress may contribute to MDS development and progression, which may be correlated with DNA methylation of tumor suppressor genes

Oxidative stress and clonal evolution: Recently, the potential of iron as a tumor promoter has been described in various types of cancer (liver, skin, GI tract, breast, oral cavity, and lung). Iron may contribute to carcinogenesis via several mechanisms triggered by ROS activity As with other tumors even in hematologic ones, this aspect has been thoroughly investigated (9). The consequences of elevated ROS in leukemia fall into two major categories: the first are the results of non-specific oxidative damage to biomolecules, the second are more specific effect arising from hyper activation of ROS signaling pathways. Thus a detailed understanding the role of ROS as key mediators in leukemogenesis may provide new instruments in prognostic and therapeutic features of hematologic disease. A clonal myeloid disorder such as

MDS is intrinsically linked to AML development and is characterized by genomic instability "per se". It is believed that oxidative stress may contribute to an AML-promoting effect since leukemic cells start from an higher ROS levels compare to a normal leukocyte. Age-induced genetic/epigenetic, and immune-mediated changes in hematopoietic stem cells lead to oligoclonal or monoclonal expansion of myelodysplastic initiating cells. These cells display a defective differentiation, without inducing proliferation, and are characterized by increased apoptosis of erythroid and myeloid progenitors, leading to cytopenias. AML evolution occurs when these myelodysplastic cells accumulate additional genetic lesions that promote proliferation. *In vitro* experiments have identified the underlying process indicated sustained DNA damage (DNA double-strand breaks) as a result of exposure to excess iron. Other models showed how increased ROS levels stimulate leukaemogenesis through the regulation of redox-sensitive transcription factors (Nrf2, Bach1, NF- kB, HIF1). There were progressive alterations of key pathways, including activation of Akt, NF- B, Wnt, antioxidant defenses, and DNA damage response, as well as inactivation of JNK, C/EBP , and PTEN (10). Unpublished data from Chan et al in a murine model showed how the highest rate of AML was observed starting at 7 months after irradiation in the group receiving the lower amount of iron-loaded (7,5mg) compared to 15 mg or 30 mg group. Their results suggest that non-lethal amounts of oxidative stress may promote mutagenesis and therefore carcinogenesis instead of cell death. The relationship between iron burden and AML risk may resemble a biphasic dose-response curve, in which the risk of AML increases up to a peak iron dose (where the proliferative aspect prevails) and then declines at higher dose (where the apoptotic aspect prevails).

Oxidative stress and dna methylation: The aberrant epigenetic landscape, including deregulated DNA methylation patterns, is a hallmark of many myeloid malignancies, including MDS. The hypermethylation of tumor suppressor genes (e.g. P15, P16, and DAPK), transcription factors (e.g. GATA1), and other genes involved in the regulation of signaling transduction pathways (e.g. ESR1, SHP1, and SOCS1) are frequent events in these neoplasms. ROS levels and DNA methylation patterns change with age. It is generally accepted that ROS levels increase with age leading to protein, lipid, and DNA damage; but also that there is a refined interrelationship between oxidative stress and DNA methylation. Similarly, the methylation pattern also changes during lifetime. The hypothesis that ROS might modulate the DNA methylation result from the fact that cancer cells display abnormal methylation patterns and are often in a state of oxidative stress. The basic idea is that that oxidative stress may contribute to MDS development and progression, which may be correlated with DNA methylation of tumor suppressor genes like the cell cycle inhibitors P15 and P16.Goncalves et al demonstrated *in vivo* that MDS patients had higher P15 and P16 methylation frequencies compared with controls. The hyper-methylation of P15 and P16 tumor suppresser genes occurs frequently in MDS patients, and these epigenetic abnormalities have been associated with disease progression and AML transformation (11). Based on these findings, it is not difficult to hypothesize an effective association of two drugs such as ironchelators and DNA-demethylating agents.

FUTURE PROSPECTIVES ROS levels detection: In daily clinical practice and in the retrospective clinical trials, iron overload is usually documented with standard markers (ferritin, blood transfusions, MRI) and tissue damage and outcomes are tentatively associated with these parameters. In 2014 Coates described iron tissue damage as the sum of iron reactive species, genetics, environmental factors and time of exposure. He postulated that serum ferritin and other iron overload markers are evidence of prolonged past tissue iron species exposure but not direct triggers of tissue damage (12). Also we learned how the scientific community has established that, depending on their concentration, ROS can trigger apoptosis or stimulate cell proliferation. Therefore the recent introduction of NTBI, LPI/LCI and oxidative stress notions influenced physicians to develop a detection method to identify these parameters in order to better understand the possibility of using biomarker in prospective clinical trials and predicting outcome. Goncalves *et al.* in 2015 showed *in vivo* that myeloid blast cells from MDS patients had increased intracellular peroxide levels and decreased GSH. Blasts, erythroid precursors and granulocytes had the highest ROS levels leading to increased susceptibility to apoptosis and DNA damage. They concluded that MDS patients with high ROS levels and low GSH levels in blast erythroid precursor and granulocytes, had shorter overall survival (13). The results of this study are significant because they give us an idea of how 'unusual parameters' such as ROS may be used to detect iron tissue damage and to predict overall survival. They also postulate that oxidative stress levels were MDS subtype- and IPSS risk group-dependent. Low-risk patients had the highest ROS levels, which can be related with their high apoptosis (cytopenia); and intermediate-2-risk patients had lower ROS levels that may be associated with their proliferative potential.

Table 2. Shows the standard and the experimental methods of iron detection.

STANDARD	EXPERIMENTAL
➢ ferritin, transferrin saturation ➢ blood transfusion intake ➢ MRI	➢ NTBI, LPI/LCI , ➢ ROS (peroxides, superoxide, peroxides/superoxide ratio) ➢ Reduced glutathione (GSH) ➢ Lipid peroxidases

ROS as drugs target: It has been recently considered that ROS could represent a potential therapeutic target. Two experimental therapeutic strategies can be applied: Administration of compounds that amplifies the existing ROS in malignant cells. This approach is expected to increase ROS level and, therefore, activate cell death. Suppression of ROS. This approach is based on the fact that ROS-generating cancers develop a new redox homeostatic state requiring higher ROS levels to survival than normal cells. These strategies, use ROS-modulating agents, induce cancer cell dead and, simultaneously, protect normal cells from oxidative damage. Understandably iron chelators remain the standard of care to reduce cellular oxidative stress. Their clinical beneficial are well known instead remain ongoing experimental trials attempting to describe the potentials of action on oxidative stress and stem cell growth. In particular, it is unknown why only part of the patients develop a clinical benefit in terms of hematological improvement and leukemia free survival. Possible associations with de-methylation agent drugs are under investigation since the relationship between iron and DNA methylation has been clarify.

Conclusions: In hematological malignancies, particularly in MDS, increased ROS production due to cellular free iron overload may overwhelm the cellular antioxidant defenses, leading to an oxidative stress state. Excess of ROS induces several DNA modifications, oxidative damage to lipids, proteins, and mitochondria. Moreover, ROS play an important role in intracellular signaling and several biological processes are dependent upon appropriate intracellular ROS levels, namely those involved in the activation of signaling pathways such as proliferation, differentiation, and cell death. High levels of ROS may contribute to cancer development through both genetic and epigenetic mechanisms. Decipher the mechanism that link ROS levels with HSC growth is essential. The aim of this effort is to try to understand how oxidative stress product can be used to predict disease progression or to develop new therapies.

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Hematopoietic stem cell transplant (HSCT) is potentially curative for a wide variety of diseases, including neoplastic and non neoplastic diseases. Choice of a stem cell donor is dependent on donor availability, donor compatibility, recipient disease and phase of disease. Historically, human leukocyte antigen (HLA)-matched sibling donors have been preferred donors for HSCT; however, only about 25% of patients will have a MSD (matched sibling donor) available. The majority of patients with an indications for HSCT will require a non sibling donor. Alternative sources of hematopoietic stem cell for transplant are matched unrelated donors (MUD), 1-antigen mismatched unrelated donors (MMUD), haploidentical donors (haplo), and umbilical cord blood (UCB) units. Despite the availability of more than 25 million potential haematopoietic stem cell transplantation (HSCT) donors in the National Marrow Donor Program donor registry showed that the probability of finding an 8/8 matched adult donor varies considerably, from 75% in Caucasians to 16% among other races[1]. Therefore, MMUD, UCB, and haplo donor graft sources expand the donor pool for recipients who do not have a MSD or MUD available. Given the variety of different donor stem cell sources available today, nearly every patient who needs an allogeneic HSCT has a potential donor in 2017. Umbilical cord blood offers the advantage of easy procurement, no risks for donors, reduced risk of transmitting infections, immediate availability and less stringent criteria for HLA matching. The disparity between patients body weight and CB cell content, particularly when associated with a two-antigen HLA mismatch, increases the risk of graft failure and delays hematopoietic and immunological reconstitution[2]. One potentially attractive alternative source of stem cells is a haploidentical relative. The haploidentical family donors offers a promptly source of hematopoietic stem cells for almost all patients[3]. Many haplo-SCT protocols have been successfully established, with promising clinical outcomes, due to improved understanding of the mechanisms underlying the HLA barriers and how to cross them[4-6]. In recent years plenty of studies have been published as comparing HSC transplant from haplo donors to other alternative donors. The validity of these papers is limited by the retrospective nature of data[7-10]. Nowadays the choice of donor, when a MSD is not available in the family, is driven by type and phase of disease to the transplant indication and by expertise and policy of each center.

In the future, we need randomized trials to compare outcomes between transplants from the alternative donor types.

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REGULATORY MYELOID CELLS IN HEMATOLOGICAL MALIGNANCIES

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Regulatory myeloid cells, largely known also as myeloid derived suppressor cells (MDSCs) comprise heterogeneous populations of myeloid cells at various maturation stage, initially identified in of tumor-bearing mice as CD11b+Gr1+ elements and based on the expression of Ly6C distinguished in granulocytic (PMN-MDSC, Ly6G+, high "side-scattered light"-SSC) and monocytic fractions (M-MDSC, (Ly6G-, low SSC). In absence of a marker equivalent to Ly6C, several MDSC subpopulations have been described in humans termed monocytic (M-MDSCs, CD11b+CD14^{+}HLA-DR$^{low/-}$) and granulocytic (PMN-MDSCs, CD11b^{+} CD14^{-} CD15^{+}HLA-DR$^{low/-}$), respectively. To avoid confusion in the field, updated nomenclature and characterization standards of MDSCs were recently proposed. The classic definition of MDSCs as immature myeloid cells that are blocked from differentiating has been challenged by recent studies that have suggested that M-MDSCs and PMN-MDSCs may represent monocytes and granulocytes with acquired immunosuppressive properties, as consequence of a cancer-driven or emergency hematopoiesis due to tumor-related cytokine release. However, it is still under investigation if this modified hematopoiesis occurs at bone marrow level or not, with important implications for hematological malignancies where often the site of emergency hematopoiesis and neoplastic cells can coincide [1]. Transcription factors crucial for emergency myelopoiesis, contributing to accumulation of MDSCs and terminal maturation of tumour-associated macrophages (TAM) are RORC-1, through its activity on modulating the myelopoietic activity of G- and GM-CSF, and IFN Regulatory Factor 8 (IRF8) [2]. In presence of tumor conditioned medium or in response to GM-CSF, bone marrow progenitors from Rorc12/2 mice failed to differentiate to macrophages and displayed increased differentiation into granulocytes, whereas treatment with G-CSF resulted in reduced granulocyte production. Moreover, PMN-MDSC infiltration was increased in irradiated chimeric mice that received Rorc12/2 hematopoietic progenitors suggesting the involvement of RORC1 to terminal differentiation and M2-polarization in macrophages [1, 2]. IRF8 promotes terminal macrophage maturation and acts as a negative regulator of MDSC expansion. IRF8-deficient mice exhibit deregulated myeloid cell differentiation and resultant accumulation of CD11b+Gr1+ MDSCs. However, when IRF8 deficiency is restricted to myeloid cells, the myeloid cell lineage differentiation is normal. On the contrary, mice with IRF8 deficiency only in T cells exhibited deregulated myeloid cell differentiation and MDSC accumulation, because IRF8-deficient T cells exhibit elevated GM-CSF expression and secretion. Treatment of mice with GM-CSF increased MDSC accumulation, and adoptive transfer of IRF8-deficient T cells, but not GM-CSF-deficient T cells, increased MDSC accumulation in the recipient chimeric mice [3], thus confirming that IRF8 regulates apoptosis in myeloid cells and represses GM-CSF expression in T cells to control myeloid cell lineage differentiation. Granulocyte-monocyte progenitor cells can be distinguished in IRF8^{high} and IRF8^{low} cells that correspond to monocytic and granulocytic progenitor phenotypes. IRF8^{low} granulocyte-monocyte progenitor cells preferentially expand with increasing tumor size and increasing G-CSF serum concentration; enforced IRF8 expression restrains this expansion and reduces the frequency of myeloid suppressors in the periphery. The term suppressive

in MDSC refers to the peculiar ability to elicit T-cell anergy due to insufficiency in co-stimulatory signaling and loss of function in the T-cell receptor. Aberrancies in specific biochemical pathways include aminoacid deprivation, like arginine, due to the high expression level of arginase (Arg-1), cysteine for aberrant expression of transporters in the immunological synapsis, tryptophan for high levels of IDO-1, reactive oxygen species (ROS) release , nytrosylation of T-cell receptor, thus contributing to immune-surveillance evasion [4, 5]. The role of MDSCs in lymphoma is only beginning to be explored, but preclinical evidence has emerged suggesting this population to be a major driver of tolerance. In the A20 lymphoma mouse model [6, 7], MDSCs induced activation and proliferation of antigen-specific regulatory T-cells (Tregs), leading to suppression and anergy of anti-tumor effector T-cells. Moreover, MDSC can up-take tumor antigen, thus to passively limit the amount of antigen that can be processed by other professional antigen-presenting cells like dendritic cells, worsening the immune-suppression. Consistent with these findings, depletion of MDSCs in a mouse model of lymphoma inhibited tumor growth. MMDSCs are the circulant precursors of TAMs and several groups have investigated their clinical usefulness in solid and liquid cancers. For many types of solid tumors, a high degree of macrophage infiltration has long been associated with a poor patient prognosis. In patients with classic Hodgkin lymphoma (cHL), a gene signature of TAMs and a high number of CD68+ cells in the tumor have been associated with shortened survival durations after treatment with chemotherapy regimens, compared with that of patients without these characteristics; therefore, TAMs have been proposed as a biomarker for risk stratification. High CD68 or CD163 expression have subsequently been confirmed to be independent predictors of unfavorable survival in the multicenter, randomized, controlled, E2496 Intergroup trial67, thus reinforcing the prognostic significance of TAMs in chemotherapytreated patients with locally extensive and advancedstage cHL. Our group showed that in cHL patients MDSC count and their surrogate Arg-1 have prognostic meaning, identifying at diagnosis poor outcome also in those patients treated up-front with a PET-2-driven approach, and that TAM are Arg-1 positive in cHL lymphonodes [8, 9]. In B-cell non-Hodgkin lymphoma M-MDSC were unresponsive to Toll-like receptor stimulation by CpG and resistant to maturing into $CD83^{+}$dendritic cells, through elevated Arg-1, impaired STAT1 phosphorylation, interferon- , and Tumor necrosis factor- receptor II (CD120b). Patients with increased ratios of M-MDSC/ monocytes had more aggressive disease and suppressed immune functions [10]. In Multiple Myeloma (MM) there is an emerging interest of MDSC to explain the complex network of cells and soluble factors in the microenvironment. In two immune-competent murine models of MM, T-cell suppressive capacity of MDSC was an early event, needed for the growth and expansion of neoplastic plasma cells [11, 12]. MDSC accumulated in spleen at later time points and to a lesser extent than in bone marrow, without any difference in the kinetics for G-MDSC or M-MDSC. While in murine models, M-MDSC were the main subset with larger immunosuppressive activity than G-MDSC, in humans, data are still under investigation. Both M- and G-MDSC subsets sorted from MM bone marrow and peripheral blood are immunosuppressive, having G-MDSC more suppressive activity than M-MDSC [13]. G-MDSC are increased in PB of MM patients and are able to induce the generation of T-reg. G-CSF administered to induce stem cell mobilization, caused an increase in the number of MDSC in the peripheral blood of patients with MM and a concentration of these immune-suppressive cells in peripheral blood stem cell collections [14]. Later, Ramachandran *et al.* showed that G-MDSC are not distinguishable from mature neutrophils in MM patients, and responsible of chemotherapy refractoriness [15]. MDSC can induce metabolic changes in neoplastic cells re-shaping the availability of aminoacids in the microenvironment. Our group showed that arginine deprivation triggered the integrated stress response via GCN2/EIF2a/ATF4 pathway, to sustain p62 and IRF4 levels, two key-molecules for MM survival and growth. Conversely, stable lentiviral GCN2 silencing abolished IRF4 synthesis, and caused the in vitro extinction of MM cell lines within 5 days of culture (Romano, unpublished data). MDSC are involved in crucial processes for active MM, such as angiogenesis, since MDSC are able to release metalloproteinase, and osteoclastogenesis, since MDSC can work as osteoclast progenitors [16, 17], thereby contributing to osteolytic bone disease in MM [18]. Our group showed that mesenchymal stem cells obtained from MM, but not MGUS, patients are able to activate G-MDSC and contribute to bone resorption [19]. Several strategies are currently under investigation in human cancer to target MDSC in order to improve immune therapies: 1) deactivation of MDSC (using phosphodiesterase inhibitors, nitroaspirins, synthetic triterpenoids, COX2 inhibitors, Arg-1 inhibitors, anti-glycan antibodies, CSF-1R, IL-17 inhibitors and histamine based approaches); 2) Differentiation of MDSC into mature cells (with ATRA, vitamins A or D3 or IL-12); 3) Inhibition of myeloid cell development into MDSC (with N-Bisphosphonates, modulators of tyrosine kinases, and STAT3 inhibitors); 4) Depletion of MDSC (using gemcitabine, HSP90 inhibitors, and paclitaxel) as recently reviewed [20]. Phosphodiesterase-5 (PDE-5) inhibitors, including sildenafil and tadalafil, inhibit the degradation of cyclic guanosinemonophosphate (cGMP) leading to reduction in ARG1and NOS2 expression, thus turning off the immunosuppressive property of MDSC [6]. In an in-vitro model, sildenafil was able to restore expansion of T cells within the peripheral blood mononuclear cell fraction isolated from MM patients [6] thus leading PDE5-inhibitors as novel immunotherapy in MM.

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DIRECT ORAL ANTICOAGULANTS AND HEMATOLOGICAL NEOPLASMS: FUTURE INDICATIONS AND PITFALLS

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Introduction: Venous thromboembolism (VTE) is a significant cause of

both morbidity and mortality in patients with cancer [1-4]. It is currently estimated that the annual incidence of VTE in patients with cancer is 0.5% compared to 0.1% in the general population [5] Active cancer accounts for 20% of the overall incidence of VTE [6]. VTE occurrence in cancer patients results from a multi-factorial risk combination of cancer-induced hypercoagulability, endothelial and vessel wall damage, and venous stasis [7,8]. Hypercoagulability is induced through pro-coagulant and inflammatory cytokines, while venous stasis can be caused by both immobility and venous external compression. Endothelial and vessel damage can occur through chemotherapy-induced interleukin and tumor necrosis factor release as well as indwelling catheters needed for medication administration. Risk for VTE and recurrent VTE is highest among patients with hematologic malignancies, lung cancer, gastrointestinal cancer (stomach/colon), pancreatic cancer, kidney cancer, and bone cancer, patients with myelodysplastic disorder, and patients with metastases. [2,8] The risk of VTE is amplified in cancer patients with concomitant risk factors such as older age, platelet count ≥350×10^9/L, hemoglobin <100 g/L or use of red cell growth factors, leukocyte count ≥11×10^9/L, BMI >35 kg/m^, inherited thrombophilia due to factor V Leiden or prothrombin 20210A mutations [2,6,7]. Finally, VTE risk is increased by chemotherapeutic agents, immunomodulatory drugs (IMIDs) (thalidomide/lenalidomide plus high dose dexamethasone), and hormonal therapies, such as tamoxifen/raloxifene, diethylstibestrol, hormone replacement therapy, and oral contraceptives [7]. Risk predictions models include the Ottawa score for recurrent cancer-associated VTE and the Khoranna score for chemotherapy-associated VTE [9,10]. Cancer patients with VTE are at increased risk for both bleeding and VTE recurrence [11]. Based largely on the CLOT and CATCH trials, all current guidelines recommend low molecular weigth heparin (LMWH) for at least 3-6 months in cancer-associated VTE, suggesting to treat indefinitely patients with active malignancy and ongoing treatment [12,13], on the basis of its overall superior safety and efficacy compared to vitamin K antagonists (VKA).

Direct oral anticoagulants (DOACs) for treatment of VTE in cancer patients: DOACs dabigatran, rivaroxaban, apixaban, and edoxaban are approved for the treatment of acute VTE, and the combined six phase-3 trials have included >1500 patients with active cancer. Subgroup analyses of these patients, either pooled or separately reported, suggest that DOACs could be a safe and efficacious alternative to VKA therapy for the treatment of cancer-associated VTE. Of 771 patients with cancer enrolled in the Hokusai-VTE trial, 378 were assigned to edoxaban and 393 to warfarin. Recurrent VTE occurred in 4% of the patients given edoxaban and in 7% of the patients given warfarin (hazard ratio [HR] 0.53, 95%CI 0.28-1.00, p=0·0007). Clinically relevant bleeding (major or non-major) occurred in 12% of the patients who received edoxaban and in 19% of the patients who received warfarin; the HR for clinically relevant bleeding was 0.4, 95%CI 0.45-0.92, p=0·017. Therefore, edoxaban was found non-inferior to warfarin for the treatment of cancer patients with VTE, and with less clinically relevant bleeding [14]. The only additional data on DOAC use in cancer patients come from pooled analyses. In one analysis, 514 patients with active cancer who were treated with a DOAC were compared to 459 treated with a VKA. The pooled incidence rate of recurrent VTE during DOAC therapy was 4.1% compared to 6.1% with VKAs (HR 0.66, 95% CI 0.38-1.2). The rate of major and clinically-relevant non-major bleeding was similar in both groups (15% *vs* 16%, RR 0.94, 95% CI 0.7-1.3) [15]. A pooled analysis examining only dabigatran therapy found similar results [16]. However, no specific data from direct head-to-head comparisons of DOACs with LMWHs are currently available. Therefore the use of DOACs for the management of VTE in cancer is thus not recommended by clinical practice guidelines. The aforementioned studies did not include a detailed definition of 'active cancer'. The more generally employed definitions are 'a diagnosis of cancer within 6 months prior to enrolment, any treatment for cancer within the previous 6 months, or recurrent or metastatic cancer"; therefore no data can be indirectly drawn for efficacy and safety in the subgroup of hematological malignancies.

DOACs for prevention or treatment of VTE in patients with leukemia: Direct thrombin inhibitors (gatrans) (e.g. dabigatran) or factor Xa inhibitors (xabans) (e.g. rivaroxaban, apixaban, edoxaban) do not rely on antithrombin (AT) for the inhibition of coagulation. This could be of great interest during the imbalance in haemostasis towards thrombosis and the special decreases in AT levels occurring during asparaginase treatment in patients with acute lymphoyd leukemia (ALL) [17]. One of these direct thrombin inhibitors, melagatran, produced *in vitro* a significant reduction in endogenous thrombin generation in the plasma of children with ALL obtained during induction with asparaginase. In contrast, the anticoagulant action of LMWH was markedly affected by endogenous AT levels [18]. The pro-drug xilamegatran, tested in clinical trials, has been withdrawn from the market because of concerns about potential liver toxicity. However, the results from the present study might be used as a model for other direct thrombin or factor Xa direct inhibitors in situations with reduced AT levels. Edoxaban, a direct factor Xa inhibitor, was proven to exert a comparable antithrombotic effect even in mice with low plasma AT, similar to that observed in wild-type mice [19]. From a clinical point of view, one completed post-hoc, sub-group analysis which specifically evaluated the use of dabigatran compared to VKAs in thrombophilia, demonstrated that dabigatran was non-inferior to warfarin in patients with thrombophilia in recurrent VTE or VTE-related deaths. In this study patients with AT deficiency were 11/262 per arm (4%) [20]. Moreover, a case of a 12-year-old girl with heparin-resistant severe thrombosis due to AT deficiency (homozygous AT Budapest III) and successfully treated with rivaroxaban has been published recently [21].

Table 1. Ongoing DOACs (apixaban) trials in patients with hematological malignancies. DVT: deep venous thrombosis; VTE: venous thromboembolism.

Trial	Clinicaltrials.gov identifier	Disease	Active arm	Comparator	Time frame	Primary outcome
A phase III randomized, open label, multicenter study of the safety and efficacy of apixaban for thromboembolism prevention versus no systemic anticoagulant prophylaxis during induction chemotherapy in children with newly diagnosed acute lymphoblastic leukemia (ALL) or lymphoma (T or B cell) treated with peg/rasn1-asparaginase	NCT02466633 (January 2015)	Lymphoma Acute Lymphoblastic Leukemia	Children assigned to the apixaban arm <35 kg will get a dose of 0.07 mg/kg twice a day with the 0.4 mg/ml solution for 25-28 days Children assigned to the apixaban arm ≥ 35 kg will either get a 2.5 mg tablet twice a day or 0.2 ml of the 0.4 mg/ml solution twice a day for 25-28 days	Placebo No systemic anticoagulant prophylaxis	1 month	A composite of adjudicated non-fatal DVT (either asymptomatic and symptomatic), pulmonary embolism, and cerebral venous thrombosis and VTE-related death Major bleeding
Evaluation of the use of an oral direct anti-Xa anticoagulant, apixaban, in prevention of venous thromboembolic disease in patients treated with IMIDs during myeloma : a pilot study	NCT02200458 (February 2014)	Multiple Myeloma	Apixaban 2.5mg x 2 per day for 6 months	Single arm	6 months	Total VTE and VTE-related death. Major and clinically relevant non-major bleeding
A pilot study investigating apixaban and dexamethasone interaction in multiple myeloma	NCT02749617 (March 2016)	Multiple Myeloma	Apixaban 2.5mg x 2 per day for 3 months	Single arm	3 months	Anti Xa activity (measure of level of prophylaxis provided by apixaban)
Apixaban for primary prevention of venous thromboembolism in patients with multiple myeloma receiving immunomodulatory therapy	NCT02459860 (November 2016)	Multiple Myeloma	Apixaban 2.5 mg x 2 per day for 6 months	Placebo	6 months	Symptomatic VTE Major and clinically relevant non-major bleeding

Therefore, DOACs were demonstrated to be effective on clinical grounds in cases where inherited AT deficiency could hamper the effect of heparin(s). At least two cases of patients with acquired AT deficiency due to asparaginase treatment have been reported. A 41-year-old man with ALL and heterozygous for factor V Leiden had a superficial thrombosis of the cephalic vein after treatment with Peg-asparaginase (AT 31%, fibrinogen 160 mg/dL, platelet count 120×10^9/L); treatment with rivaroxaban 15 mg×2 for 13 days resolved completely symptoms [22]. A 22-year-old man with ALL was diagnosed with superior sagittal sinus thrombosis due to Peg-asparaginase (AT 57%, fibrinogen 77 mg/dL). He received cryoprecipitate and AT concentrate (target AT level 80%); heparin infusion was transited to apixaban 10 mg×2 for 7 days, followed by 5 mg×2 [23]. A phase III, multicenter, international, open-label, study has been launched, that randomizes pediatric patients (ages 1 to <18 years) with ALL or lymphoma treated with asparaginase to either the placebo group or the intervention group (Table 1). Patients who are 2 years old and above weighing less than 35 kg are given 0.07 mg/kg twice daily of apixaban as a 0.4 mg/ml solution and patients weighing ≥35 kg are given a 2.5-mg tablet twice daily. The primary endpoint of this study is a composite of non-fatal deep venous thrombosis, pulmonary embolism, cerebral venous sinus thrombosis, and VTE-related death up to 1 month after therapy. The researchers are planning to recruit a total of 700 subjects. If this recruitment is successful, this will be the first adequately powered phase III clinical trial evaluating DOACs in the pediatric population [24].

DOACs for prevention or treatment of VTE in patients with multiple myeloma: Multiple myeloma is associated with an increased risk of VTE. Risk factors can be patient related (advanced age, other risk factors shared with the general population), disease related, and treatment related. Disease-related risk factors can derive from the monoclonal component (rarely hyperviscosity or inhibition of natural anticoagulants) or hypercoagulability sustained by inflammatory cytokines (increased von Willebrand factor, factor VIII, fibrinogen levels, decreased protein S levels, acquired activated protein C resistance) [25]. The 1 to 2% baseline of incident VTE associated with conventional therapies as melpha-

lan and prednisone is at least doubled by the use of doxorubicin or other chemotherapeutic agents. The VTE rate associated with thalidomide or lenalidomide as monotherapy is similar, whereas combination with high-dose dexamethasone or multiple chemotherapeutic agents induces a multiplicative effect on the VTE rate up to 25%. LMWH, fixed low-dose warfarin, and aspirin are acceptable strategies for antithrombotic prophylaxis, reducing VTE to 5 to 8% in thalidomide-treated patients and 1 to 3% in lenalidomide-treated patients [25]. In a survey conducted in Ireland, most physicians involved in treatment of patients with multiple myeloma employed LMWH or aspirin (82% and 71%, respectively). However, 3 of 28 (11%) had used dabigatran or rivaroxaban despite there was little evidence to support their use [26]. One population-based study extracted data from the French health insurance scheme database (SNIIRAM, Système National d'Information Inter-Régime de l'Assurance Maladie) for the Midi-Pyrénées area (South West France, 2.8 million inhabitants). In a cohort of 236 patients with multiple myeloma newly diagnosed from January 2012 to September 2013, 77 (32.6%) received antithrombotic prophylaxis for VTE: the most represented drugs were aspirin, LMWH, and VKA; only one patient (1.3%) received rivaroxaban [27]. Case reports concerning the use of DOACs in patients with multiple myeloma are anecdotal. Effective treatment with apixaban 10 mg/day has been reported in one 69-year-old man with relapsed multiple myeloma and VTE occurred during a course of lenalidomide and low-dose dexamethasone [28]. In the MYELAXAT trial (Table 1) [29] myeloma patients requiring Melphalan-Prednisone-Thalidomide in first line, or Lenalidomide-Dexamethasone in relapse were enrolled between 2014 - 2016. All patients received apixaban, 2.5 mg×2/day for 6 months as primary prophylaxis for VTE. Out of 104 patients, two VTE events were registered, i.e an asymptomatic proximal deep vein thrombosis (DVT) and a symptomatic distal DVT; in the latter case, apixaban was stopped 14 days before. Only one major bleeding was reported. Thus, apixaban used in a preventive scheme seems to be efficient and safe in preventing VTE in myeloma patients treated with IMIDs [30].

DOACs for treatment of VTE in patients with Philadelphia-negative myeloproliferative neoplasms: In patients with myeloproliferative neoplasms VKA treatment is highly effective in preventing recurrent VTE [31-33]. In a cohort of 206 patients with MPN-related DVT the overall incidence rate of recurrent thrombosis was 4.7 per 100 pt-years on VKA and 8.9 per 100 pt-years off VKA (p=0.03); consistently, the incidence rate of recurrent VTE was 3.7 per 100 pt-years on VKA and 7.1 per 100 pt-years off VKA (p=0.04). Discontinuation was associated with a 2.2- fold increased risk of novel thrombotic events over time. The incidence of major bleeding was 2.4 per 100 pt-years on VKA and 0.7 per 100 pt-years off VKA [33]. MPN patients are prone to either thrombotic and haemorrhagic increased risk, and in this setting the use of DOACs could ameliorate the bleeding risk. In the aforementioned cohort of MPN patients with DVT only 3.3% of patients were treated with DOACs [33]. In a single center registry of 760 MPN patients, 25 (3.3%) were treated with a DOAC. The reasons for prescribing DOACs were atrial fibrillation and thrombotic events for 13 and 12 patients, respectively [34]. In the German MPN registry of the Study Alliance Leukemia, 68 out of 454 patients (14.9%) had suffered from DVT or splanchnic vein thrombosis; only 8 patients (1.7% of the cohort) were treated with rivaroxaban. However, multivariate analysis revealed an odds ratio of major bleeding for patients on rivaroxaban of 1.61 (non-significant), which is lower than those for patients on VKA therapy (1.97), double platelet inhibition (3.05) and heparin (5.64, the only drug with a significant odds ratio for major bleeding) [35].

Conclusions: The use of DOACs in the primary and secondary prophylaxis of VTE in patients with haematological malignancies is promising. As in the general population, the risk-benefit profile seems favourable in comparison with VKA, with a similar efficacy in the treatment of VTE and a reduced risk of major bleeding. A clinical scenario where the exploratory use of DOACs is particularly attractive is prevention or treatment of VTE in AT-depleted patients treated with asparaginase, taking benefit of the direct action on the target factor II or factor×molecules without need of the AT pathway. However, some limitations in the field of malignant hematology can be anticipated. Patients on DOACs do not need routine coagulation monitoring, but in some challenging patients with hematologic malignancies drug titration could be necessary, such as patients with chemotherapy-induced or disease-related thrombocytopenia where anticoagulation is at high risk of bleeding [36]. In this setting LMWH dose-reduction according to the platelet count has been proposed by some experts [37,38], but the adoption of a similar strategy for DOACs is quite unexplored. The dosing regimen and dose adjustments for renal impairment are DOAC specific and different from each other and thus predispose to dosing and prescription errors in patients with renal impairment, as those with multiple myeloma. Finally, the relative lack of drug interactions in DOACs is an advantage but clinicians must be aware of the few important drug interactions with some antifungal, anti-microbial and anti-viral medications involving the Cytochrome P450 3A4 and the P-glycoprotein metabolic pathways [39]. In conclusions, controlled clinical trials targeted to patients with haematological neoplasms are urgently needed to explore advantages and pitfalls of novel DOACs in this special cancer setting.

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WHO 2016: WHAT CHANGES FOR LYMPHOID NEOPLASMS

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The forthcoming update of the 4th WHO classification of lymphoid tumours is expected by the end of 2017 as revised monograph. It will maintain its structure which proved of great help in the diagnosis of well-established entities and identification of uncommon lymphomatypes. The update will refine already known definite lymphoma categories, will confirm as definite categories termed provisional in the 2008 version, while other lymphoma types will be proposed as provisional. Below some of the most significant changes will be briefly commented.

B-cell Lymphomas (BCL): With the availability of biologic drugs capable of interphering with the signalling pathways active in the neoplastic cells, therapies have dramatically changed in the last years. In this context, the group of Diffuse Large B-cell Lymphomas (DLBCL) are not an exception and the definition of their Cell-Of-Origin (into germinal-centre or non germinal centre/activated B-cell) has become mandatory for prognostication and therapy. This can be at present defined by means of immunohistochemistry or gene profiling, with the former being less reproducible but more widely accessible, the latter more reproducible but neither widely validated nor widely spread in laboratories. The impact of the translocations of the MYC@, BCL2@ and/or BCL6@ genes on the outcome of DLBCLs has led the recognition of the new category of "high grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangement" commonly known as "double/triple hit" LBCL. Subsequently, the categorization of "non DH/TH" LBCLs is mainly based on cytological details and phenotype. In this context the category of "B-cell lymphomas, unclassifiable with features intermediate between Burkitt and Large B-cell lymphomas" no longer exists. Within the group of aggressive B-cell lymphomas, cases with the morphological features of a Burkitt lymphoma may be kept apart if negative for the MYC@ translocation. At least some of them may harbour chromosome 11q alteration characterized by proximal gains and telomeric losses; Though of rare occurrence, these cases seem to show more cytological pleomorphism, lower MYC protein expression, occasionally a follicular pattern, frequent nodal presentation and a more complex karyotype. The possibility of diagnosing an EBV positive LBCL has been expanded to patients younger than 50 years of age, providing no causes of immunedeficiency are present. The category of Follicular Lymphomas has been significantly expanded, with more detailed definition of the "paediatric-type FL" and the introduction of the provisional category of "Large B-cell Lymphomas with IRF4 rearrangement" (high grade lymphoma, either follicular or diffuse, lacking t(14;18) and harbouring IRF4 gene translocation). This category occurs in young adults, at limited stage (cervical and/or tonsillar sited) and bear favourable outcome when treated. The heterogeneity of the clinical presentation of mantle cell lymphomas (MCL) has been at least in part clarified with the recognition of the "leukemic non nodal" cases which clinically and morphologically resemble splenic marginal zone lymphomas, but are provided with t(11;14) translocation; peculiar pathologic features comprise less complex karyotype, highly mutated immunoglobulin variable heavy chain genes and negativity for the SOX11 nuclear protein which is usually positive in otherwise classic MCL.

T-cell lymphomas (TCL): The recognition of a T-follicular helper phenotype and common genetic features (recurrent abnormalities in TET2, IDH2, DNMT3A, RHOA, and CD28) has allowed to gather under the same putative cell-of-origin, three types of peripheral T-cell lymphomas: angioimmunoblastic, follicular (previously recognized as a variant within the PTCL,NOS category), and cases of peripheral T cell lymphoma, not otherwise specified with TFH phenotype. The latter two are provisional entities. Among nodal aggressive T-cell lymphomas the ALK negative variant of anaplastic large cell lymphomas has been appointed as a *definite* category. Recent research advances have shown possible perspectives for risk stratification of such patients; those bearing rearrangements at the locus containing DUSP22 and IRF4 in chromosome 6p25 seem to behave more favourably, whereas the few ALK negative ALCL with TP63 rearrangements have a very aggressive clinical course. The newly recognized cases af ALK negative ALCL arising in breast implants has been included as provisional. Saline and silicone-filled implants have been implicated; the prognosis largely depends on the degree of capsule infiltration by the neoplastic cells: in case they are confined to the seroma fluid and no capsule invasion is observed the prognosis is excellent and the implant and capsule removal is the only conservative approach. Conversely if the capsule is infiltrated a systemic chemotherapy is warranted. An interesting addition in the 2017 revised version is represented by the inclusion of the so called "indolent CD8+ T cell lymphoproliferations": provisional variants occurring in the gastro-intestinal tract and at acral sites are recognized for which an aggressive therapeutic approach should be avoided. Within the aggressive T-cell lymphomas primarily arising in the gastro-intestinal tract, changes has been made within the category of "enteropathy associated PTCL": the type I form (designated as "enteropathy associated TCL" in the update), is linked to celiac disease and more commonly observed in northern European individuals, while type II-EATL will be designated as "monomorphic epitheliotropic intestinal TCL" (MEITL). It shows no association with celiac disease and more commonly occurs in Asians and Hispanic populations. The two entities also diverge as far as cellular composition and phenotype, although exceptions exist, particularly regarding the alpha/beta or gamma/delta derivation (EATL is usually alpha/beta, MEITL is usually gamma/delta but T-cell receptor silent or alpha/beta cases are reported).

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TREATMENT OF NEWLY DIAGNOSED MULTIPLE MYELOMA: GOALS AND OPTIONS

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Introduction: The treatment landscape of multiple myeloma (MM) has dramatically evolved over the last decades, with an increasing availability of highly active new therapies which have considerably improved patient outcomes. The first major change was the introduction of autologous stem cell transplantation (ASCT) in the 90'. Moreover, the understanding of the complex system of interactions between myeloma cells and the bone marrow microenvironment has further expanded the treatment options, through the introduction of new drugs targeting the MM clone in the bone marrow *milieu*. These remarkable therapeutic advances have led to a radical switch of treatments paradigms, providing the opportunity to enhance the rate of complete response (CR) and prolonging median overall survival (OS) by 3- to 4-fold. Availability of new classes of novel agents, including proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs), and use of these agents in combinations have ultimately led to higher response rates and deeper responses in the vast majority of patients with newly diagnosed and relapsed/refractory MM. Ongoing clinical trials incorporating second generation PIs and IMiDs, as well as monoclonal antibodies (mAb), are actually being explored as possible treatment options for MM in the near future. Optimal upfront therapies continue to evolve and their choice is dictated by the intent to transplant, age and comorbidities.

Goals of treatment: Unprecedented levels of genetic heterogeneity and genomic instability characterizing MM have been defined, as well as clonal evolution underlying the progression of the disease. According to this model based on the random acquisition of genetic hits and darwinian selection, clonal tides may evolve during the course of MM, and shift in dominant and sub-dominant clones with variable response to therapy and possible selection of minor drug-resistant clone(s) that may ultimately become lethal [1]. The coexistence of multiple clones with variable drug sensitivity raises the rationale for the use of combination therapy and provides the basis of continuous exposure to suppressive therapy. Moreover, the concepts of minor drug-resistant clones that may become lethal emphasize the importance of achieving and maintaining the best possible response early in the course of the disease. Indeed, extensive data indicate a clear association between the depth of response and long-term survival outcomes. Based on these data, primary goals of treatment are to maximize the depth of response, to minimize the burden of residual tumor cells and to prevent or delay relapse as a way to prolong progression free survival (PFS) and untimely OS. The integration of combination therapies and the use of sequential treatment blocks - including induction, intensification, consolidation, and maintenance - is aimed at targeting multiple clones and keeping them under control as long as possible. Current therapies leading to unprecedented rates and depth of response, have requested the development of new tools for the detection of minimal residual disease (MRD). Next generation flow and sequencing methods to quantify MRD in the bone marrow with sensitivities in the range of 10^{-5} 10^{-6} cells are currently in use. These technologies may be combined with functional imaging techniques to detect MRD outside of bone marrow [2]. Longer PFS and OS have been consistently reported for patients who have achieved MRD negativity, regardless of the risk of patients and the type of treatment. Therefore, eradicating the different tumor clones and potentially curing the disease should be considered the actual treatment end points for the management of transplant-eligible and elderly fit patients, though additional trials are needed to determine if changes in treatment are need based on MRD status. On the other hand, a disease control approach should be applied for elderly frail patients, for whom settling for a lower degree of response may be reasonable as treatment-related toxicities could outshine any benefit derived from the achievement of high quality responses. Recent developments have focused on identifying these vulnerable patients through geriatric assessment and novel MM scoring system, including the notions of frailty, disability and comorbidities [3]. This frailty score could predict survival and toxicity, as the frail patient population usually displays an increased risk of death, progression, non-hematologic adverse events and treatment discontinuation, regardless of ISS stage, chromosome abnormalities and type of treatment. Thus, maintaining a good quality of life along with avoiding treatment-related complications have become important aims in elderly patients, even if possible prolongation of OS remains the ultimate goal. Proper identification of fit and frail patients allows avoiding their possible undertreatment or overtreatment.

Transplant eligible patients: Patients aged ≤65-70 years and without significant comorbidities are candidates for ASCT. The current treatment paradigm for ASCT-eligible MM patients includes sequential blocks of therapy delivered as an induction phase, followed by intensification (ASCT), consolidation, and a maintenance phase. As previously emphasized, the objectives are to maximize the rate and duration of MRD negativity [4]. Patients typically receive a limited number of cycles of induction therapy to reduce tumor cell mass before collection of autologous hematopoietic stem cells. Novel agents, including IMiDs and PIs, combine well with traditional therapies and with one another to form various doublet and triplet regimens which have yielded unprecedented rates of high-quality responses, an early predictor of favourable outcomes following ASCT. An integrated analysis of three different randomized phase III trials has shown the superiority of bortezomib-based *versus* non-bortezomib-based induction regimens, in terms of high-quality response rates and extended PFS. Moreover, the addition of a third agent to the bortezomib-dexamethasone (VD) backbone, such as thalidomide (VTD), doxorubicin (PAD), or cyclophosphamide (VCD), resulted in improved outcomes over doublet regimens. Based on these results, VTD has been approved by the European Medicines Agency (EMA) and is listed as one of the preferred upfront option in preparation for ASCT in several guidelines [5].

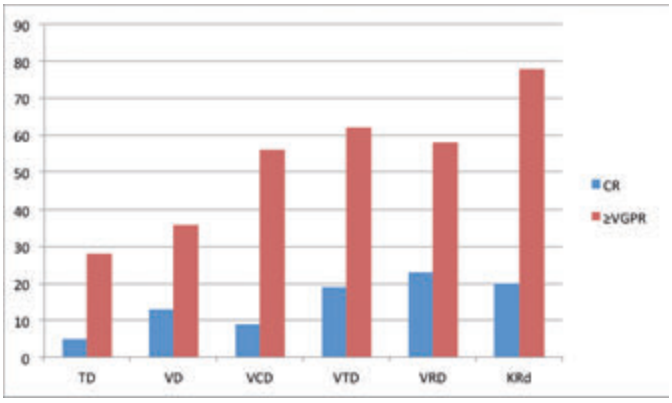

Figure 1. Response rates after novel agents induction regimens in newly diagnosed transplant eligible Multiple Myeloma patients

CR: complete response; VGPR: very good partial response; TD: thalidomide-dexamethasone; VD: bortezomib-dexamethasone; VCD: bortezomib-cyclophosphamide-dexamethasone; VTD: bortezomib- thalidomide-dexamethasone; VRD: bortezomib-lenalidomide-dexamethasone; KRd: carfilzomib- lenalidomide-dexamethasone.

One randomized trial showed that VCD and PAD were equally effective in terms of response, with more favorable toxicity profile for VCD. Moreover, based on both a retrospective case-matches analysis and a prospective trial, VTD resulted a more effective regimen compared with VCD in terms of high-quality response rates, despite a higher rate of peripheral neuropathy. VD plus the second generation IMiD lenalidomide (VRD) is likely to become a new standard induction therapy in the next few years, based on efficacy data and a lower neurotoxicity profile compared with VTD. Furthermore, preliminary data of phase II trials indicate high rates of deep responses with triplet regimens including the second generation PI carfilzomib plus ASCT, with MRD negativity rates averaging 70-80% at the end of the whole treatment program. Response rates after novel agent-based induction regimens are shown in Figure 1. The observed benefits of newer therapies incorporating the novel agents have raised questions about the role and timing of ASCT in the initial treatment of young and fit patients. Two recent phase III trials (EMN02/HO95 MM, and IFM 2009) comparing ASCT *versus* a "standard dose" intensification phase, in the context of a triplet bortezomib-based induction therapy, have shown that high-dose therapy translates into prolonged PFS, proving that ASCT should remain a standard of care even in the current era [6-7]. Another important question to be addressed in the era of novel agents includes the role of single *versus* double ASCT. In a retrospective analysis of three phase III European studies, double ASCT resulted in longer PFS and OS than a single ASCT in patients failing to achieve CR after a bortezomib-based induction therapy and carrying del(17p) and/or t(4;14). Prelimi-

nary results of the EMN02/HO95 MM trial designed to randomly assign patients to single or double ASCT in centers with a double ASCT policy confirmed the superior outcomes afforded by double ASCT in the high-risk cytogenetic subgroup. Despite these important results, a fraction of patients fails to achieve CR after ASCT. Consolidation therapy is typically short-term and is aimed at further increasing the rate and depth of response after high-dose therapy as a way to improve clinical outcomes. Similarly to the induction phase, the novel agents have been successfully incorporated into newer consolidation regimens. Preliminary results of the EMN02/HO95 MM trial showed extended PFS for patients randomized to receive a consolidation therapy with VRD *versus* no consolidation. Conversely, no benefit with consolidation treatment has been reported in the STAMINA trial designed to randomize patients to receive either a single ASCT followed or not by consolidation therapy or double ASCT, with lenalidomide maintenance in all treatment arms. Major unconsistencies between these two studies hamper any possible comparison and efforts to reconcile conflicting results. Finally, the goal of maximizing and sustaining responses has provided the rationale of "maintenance treatment", whereby continuous therapy is given to keep under control residual clonal subpopulations and to kill myeloma stem cells coming into cell cycle. Potential benefits of continuous therapy might include prevention of further evolution of the disease by restricting clonal population, further cytoreduction, maintenance of tumor suppression, prolonged duration of response and improved long-term outcomes. Conversely, potential limitations might include selection of treatment-resistant clones, quality of life, and limitation for subsequent therapy choices. A recent meta-analysis of three large phase 3 studies comparing lenalidomide *versus* placebo as maintenance therapy after ASCT demonstrated that lenalidomide maintenance was associated with an OS benefit of more than two years and has led to recent EMA approval of lenalidomide in this setting. The benefit of lenalidomide maintenance seems to be questionable in patients with a high-risk cytogenetic profile. Alternative maintenance regimens including bortezomib alone or combined with IMiDs might improve the outcomes of high-risk disease, although randomized trials designed to prospectively address this question are still lacking.

Transplant ineligible patients: Conventionally, the choice of treatment reflects a balance between priorities of efficacy *versus* tolerability, although newer combinations and schedules increasingly allow patients to benefit from the efficacy of treatment without compromising tolerability. On the basis of randomized phase III trials, the current standards of care upfront in elderly MM patients ineligible for ASCT are bortezomib-melphalan-prednisone (VMP) or lenalidomide plus low-dose dexamethasone (Rd) [5]. In the VISTA trial [8] VMP, including a twice-weekly intravenous bortezomib infusion, was proven superior to MP in response rate, CR rate, median time to progression (TTP) and OS. This superiority was sustained after a median follow-up of 60 months, in terms of median time to second-line therapy (31 months with VMP *versus* 20.5 months with MP) and median OS (56 months *versus* 43 months, respectively). Neuropathy was the major side effect of this regimen. Changes in dosing schedule (once a week) and subcutaneous administration of bortezomib have successfully reduced the rate and severity of neurological toxicity. It has been shown that a higher cumulative bortezomib dose is associated with improved OS. More recently, the FIRST trial [9] demonstrated the superiority of continuous Rd therapy over Rd or MP-thalidomide (MPT), both given for a fixed duration of 72 weeks, in term of extended PFS, the primary study end point. PFS favored Rd continuous over MPT in the majority of patient subgroups, although a questionable benefit was seen in patients with a high-risk cytogenetic profile or a high level of lactate dehydrogenase. Median PFS was prolonged in patients who responded to Rd continuous *versus* MPT, particularly in those who achieved a deeper response (≥ very good partial response, VGPR). Rd continuous extended the median time to next treatment (TTNT, 36.7 months *versus* 26.7 months) compared with MPT, and median TTNT was longer in patients who achieved CR/VGPR with Rd continuous *versus* those with MPT. Moreover, an OS benefit was reported in the continuous Rd arm *versus* MPT (59 months *versus* 49 months, respectively). The FIRST study thus established continuous Rd as a new, all oral, standard of care and alkylator-free regimen for elderly patients with newly diagnosed MM. If VMP and Rd are now considered the two most effective regimens in the first-line treatment of elderly MM patients, one way to further improve outcome might be to combine both IMiDs and PIs into the same treatment schema.

Table 1. Results of recent randomized studies in newly diagnosed transplant-ineligible Multiple Myeloma patients.

Trial	Regimen	≥VGPR (%)	PFS (median, months)	p	OS (median, months)	p
VISTA	MP	8	16.6	<0.001	43	<0.001
	VMP	41	24.0		56	
FIRST	MPT	28	21.2	<0.001	49	0.016*
	Rd x 18 cycles	43	20.7		57	
	Rd continuous	44	25.5		59	
SWOG S0777	Rd	31	30.0	0.002	64	0.025
	VRd	44	43.0		75	

**Rd continuous versus MPT. VGPR: very good partial response; PFS: progression free survival; OS: overall survival; MP: melphalan-prednisone; VMP: bortezomib-melphalan-prednisone; MPT: melphalan-prednisone-thalidomide; Rd: lenalidomide-dexamethasone; VRd: bortezomib-lenalidomide-dexamethasone.*

The GEM2010MAS65 trial, compared a sequential arm consisting of 9 cycles of VMP followed by 9 cycles of Rd, to an alternating arm consisting on one cycle of VMP alternating with one Rd, up to 18 cycles, in elderly MM patients with newly diagnosed MM. These two approaches were both very effective, and no difference was seen between the two arms: median PFS 32 and 34 months, median OS not reached, and 3-years OS was 72 and 74%. Moreover, MRD evaluation identified a subgroup of patients with MRD negativity who had an excellent outcome. Lastly, up-front use of triplet regimens based on the synergistic effect of lenalidomide and bortezomib has recently been explored in the phase III randomized trial SWOG S0777, for patients without an immediate intent for ASCT. Median PFS was significantly improved in the VRd group *versus* Rd group (43 months and 30 months, respectively). The median OS was also significantly improved for the VRd arm as compared to Rd (75 months *versus* 64 months, respectively). VRd remains superior to Rd for PFS and OS when adjusted for age [10]. Based on these results, VRd regimen might become a new standard treatment for transplant ineligible patients in the next few years. Results of recent randomized studies in newly diagnosed transplant-ineligible MM patients are provided in Table 1.

Future directions: The effectiveness of induction therapy and the progressively increased depth of response afforded by subsequent treatment phases correlate with extended survival outcomes. Ongoing randomized clinical trials for ASCT-eligible have been designed to explore the role of triplet regimens incorporating the second generation PIs carfilzomib or ixazomib, and four-drug regimens including mAbs targeting CD38 or SLAMF7 antigens combined with PI and an IMiD as both induction and consolidation/maintenance therapy. In the setting of transplant ineligible patients ongoing clinical studies are aimed to explore either Rd or VMP as the backbone of newer therapies incorporating the oral second generation PI ixazomib or mAbs, or a mAb combined with an IMiD plus PI-based regimen. Lastly, the understanding of disease heterogeneity and the availability of new effective therapeutic options, open the possibility of future treatment algorithms tailored to the risk stratification and MRD data.

Conclusions: The treatment landscape in MM is constantly evolving. The main goal of treatment is to achieve and maintain the best possible response early in the course of the disease. In elderly patients is also equally important balancing efficacy and toxicity. Standard treatment for transplant eligible patients include a three-drugs PI-based combinations induction therapy, ASCT, consolidation and a maintenance phase. ASCT should remain as standard of care in 2017. Tandem ASCT may be of use, particularly in high-risk patients. In the setting of non-transplant eligible patients, VMP and the all oral, alkylator-free, continuous Rd regimen are currently recommended. Recent data have shown the benefits of VRD over Rd. Newer treatments on the horizon include various combinations of the next generation PIs carfilzomib or ixazomib and the mAbs to form three- or four-drug regimens for ASCT-eligible and -ineligible patients. Finally, ongoing and planned clinical trials will help to determine whether a more individualized approach can be developed.

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ROLE OF HIGH-DOSE CHEMOTHERAPY AND AUTOGRAFT IN LYMPHOMA FOLLOWING THE INTRODUCTIN OF NOVEL TARGETED DRUGS

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Over the past decade, major advances in the treatment of lymphoma have been achieved, in particular in B-cell non-Hodgkin Lymphoma (B-NHL) and Hodgkin's Lymphoma (HL). This is due to the introduction of several targeted drugs in the clinical practice, including monoclonal antibodies, B-cell receptor signaling inhibitors, immunomodulatory agents, check-point inhibitors, and proteasome inhibitors (1). These remarkable developments, have challenged the therapeutic role of chemotherapy, in particular the use of intensive treatments delivered with autologous hematopoietic stem cell transplantation (auto-HSCT). Nevertheless, auto-HSCT remains an important therapeutic option in distinct clinical conditions in both aggressive and indolent B-NHL as well as in HL. Hereby, we will discuss the role of high-dose chemotherapy and auto-HSCT in the treatment of B-NHL and HL in the era of novel targeted agents. This short review will cover the potential use of auto-HSCT in two different clinical scenarios: i. upfront treatment in high-risk B-NHL lymphoma patients and ii. salvage therapy in patients with primary refractory or with relapsed Diffuse Large B-cell Lymphoma (DLBCL), Follicular Lymphoma (FL) and HL.

Front-line auto-HSCT in high-risk lymphoma: For long-time, intensified chemotherapy with auto-HSCT has been proposed as an effective option to improve outcome in patients presenting with high-risk clinical features. This strategy has been extensively explored in DLBCL and in FL. Based on the IPI-prognostic score, patients identified at "high-risk" had a poor outcome with CHOP-like regimens. Nevertheless, controversial results have been reported with auto-HSCT and the real efficacy of its upfront use could not be definitely proved. Following the introduction of Rituximab in combination with chemotherapy, the overall outcome of lymphoma patients has been markedly improved. At the same time, the addition of Rituximab significantly improved the therapeutic efficacy of auto-HSCT (2). Thus, the preferential use of auto-HSCT in the upfront therapy for high-risk DLBCL and FL remained a relevant issue. A few recent multicenter randomized trials, performed at multicenter level in Italy, has provided some conclusive results. The first randomized study was performed in very-high risk FL patients. Preliminary results of this study have demonstrated that upfront auto-HSCT supplemented with Rituximab did not offer any survival advantage compared to CHOP-based chemo-immunotherapy alone (3). This observation has been confirmed in a recent update of the study, as detailed in *Table 1*. Interestingly, long-term follow-up analysis of enrolled patients has shown that a non-negligible proportion of high-risk FL may experience a prolonged survival in the absence of any clinical or molecular sign of disease recurrence.

Table 1. Update on the long-term outcome in high-risk Follicular Lymphoma patients managed in the randomized trial comparing CHOP-R vs. R-HDS with auto-HSCT as primary treatment.

Parameter	All patients[1] *n*= (%)	CHOP-R *n*= (%)	R-HDS *n*= (%)
No. of patients	134	66 (49)	68 (51)
Alive patients *(median f.u. 13 years)* [2]	88 (66)	46 (70)	42 (62)
deaths for:			
➢ Lymphoma	22 (16)	13 (20)	9 (13)
➢ Toxicities[3]	20 (15)	6 (9)	14 (21)
➢ Other causes	4 (3)	1 (2)	3 (4)

[1]high-risk FL patients were enrolled in a trial comparing CHOP vs HDS and auto-HSCT, both supplemented with Rituximab. (see Ref. 3, with details of the treatment schedules). [2]Patients have been followed at long-term, with an overall median follow-up of 13 yrs. [3]Toxicities include any treatment-related fatal complication occurring early or at long-term distance.

This was more frequently seen among R-HDS treated patients compared to CHOP-R treated patients. Similarly, in high-risk DLBCL, two randomized studies, again performed by Centers in Italy, have recently documented that upfront intensified chemo-immunotherapy with auto-HSCT does not significantly improve overall survival in high-risk DLBCL, identified by the IPI score (4,5). Whether upfront auto-HSCT can improve the outcome of biologically defined poor-risk DLBCL, such as ABC-derived or Double-hit lymphoma, remains to be elucidated, as discussed in another section in this issue of the Journal (see: Pileri S et al, Diffuse Large B-Cell Lymphoma: the relevance of Gene Expression Profiling). Considering the relative rarity of these molecularly distinguishable DLBCL subtypes, large collaborative studies either registry-based or prospectively defined are needed to further outline the role of upfront auto-HCT in genetically poor-risk DLBCL. At present, the available data from randomized trials do not support the upfront use of auto-HSCT in FL and DLBCL patients, even in case of high-risk clinical presentation. The single exception to this conclusion is in primary CNS aggressive lymphoma. In this peculiar form of aggressive lymphoma, auto-HSCT remains a valuable option as upfront consolidation therapy in patients achieving disease response following HD-methotrexate and/or HD-Ara-C (6). Besides FL and DLBCL, the role of auto-HSCT in first-line therapy has been extensively investigated in Mantle Cell Lymphoma (MCL). Current evidence from the literature suggests that aggressive upfront therapies including auto-HSCT may improve the outcome in younger patients (7). A seminal study from Gianni and coworkers demonstrated in early 2000s that long term remission and cure can be achieved in MCL patients with the use of high-dose sequential chemotherapy including auto-HSCT as first line treatment approach (8). A subsequent randomized trial demonstrated the superiority of auto-HSCT consolidation over interferon maintenance in patients in Complete Response (CR) or in partial response (PR) after induction therapy (9). Multiple subsequent studies evaluating auto-HSCT as first line consolidation in MCL suggest that this should be the first choice in fit patients responding after induction chemo-immunotherapy (1). In fact, auto-HSCT is currently the standard of care in young fit patients in first CR after induction therapy containing high dose cytarabine (10). However, it should be mentioned that the role of ASCT performed in CR after induction therapy has not been prospectively evaluated in dedicated trials. During the last two decades, studies of auto-HSCT not only have shown improved overall outcome for MCL, but also offered the opportunity of evaluating the role of minimal residual disease (MRD) and *in vivo* purging in lymphoma therapy. Our group first demonstrated the feasibility of MRD monitoring in MCL and that autografting with MRD negative harvest was associated with prolonged clinical and molecular remissions (11). In a subsequent study, the feasibility and efficacy of *in vivo* purging with Rituximab was clearly demonstrated in MCL (12). Consistent with these data Rituximab proved to be effective also in molecular relapses of MCL (13). In summary, auto-HSCT has stimulated the development of molecularly-tailored treatment and at present current treatment algorithms include auto-HSCT as part of the first line therapy for young fit MCL patients in remission. Future studies should be aimed at re-evaluating the role of front line auto-HSCT given the availability of novel therapies, including anti B cell receptor or anti BCL-2 directed therapies, that appear of particular efficacy in MCL (14, 15).

Auto-HSCT as salvage treatment - I. primary refractory DLBCL: Despite improvements in treatment outcome achieved by the available treatments, a proportion of lymphoma patients displays very poor or transient response to primary treatment. This is called primary refractory disease (PRD) and it includes both patients with stable or progressive disease under primary treatment and those having a very transient response, soon followed by disease progression, within few months since treatment conclusion. At present, primary refractory disease remains a main challenge in the management of malignant lymphoma. We have recently performed a large survey on more than 3,000 lymphoma patients, managed before and after Rituximab introduction in the clinical arena (16). The overall incidence of PRD among 2.839 patients with B-cell lymphoma was of 20%. However, as shown in *Figure 1*, the incidence of PRD was significantly lower (13%) in patients receiving Rituximab-based induction treatments, compared to 27% of PRD in the pre-Rituximab era. In spite of this improvement, the incidence of PRD remains relatively high in DLBCL. Overall, 117 (15,2 %) patients displayed primary refractoriness among 765 patients treated with chemo-immunotherapy at induction. Those PRD patients had a very poor outcome, with median survival of 0.9 and 2.0 yrs, in patients with fully refractory disease and with early disease progression, respectively. Thus, even with the advent of Rituximab, early failure to primary induction therapy, with fully unresponsive or early progressing disease, remains a main clinical problem in the management of lymphoma, particularly in DLBCL. In this section we will focus on the use of salvage auto-HSCT in DLBCL, since primary treatment failure is more frequent in this subtype. Novel drugs have not shown so far special activity in DLBCL patients with PRD. For these patients, there are no effective treatment options and therapy intensification with auto-HSCT remains an opportunity to be considered at least in younger patients. Several recent reports on the use of auto-HSCT in DLB-CL patients with early induction failure showed controversial results. The validity of auto-HSCT in DLBCL patients failing rituximab-based first-line chemo-immunotherapies has been well addressed in the Collaborative Trial in Relapsed Aggressive Lymphoma (CORAL) study (17). In the CORAL trial data, DLBCL patients, treated with rituximab-based first-line chemo-immunotherapies and either not achieving CR or experiencing a relapse within 1 year of initial diagnosis had an extremely poor prognosis with salvage approaches (3-year PFS around 20%) (17). The disappointing outcomes of DLBCL patients experiencing early induction failure in the CORAL study have led several groups to question the utility of auto-HSCT in this particular setting (18). Other studies, including a registry-based report from the observational database of the Center for International Blood and Marrow Transplant Research (CIBMTR), have stressed the heterogeneity of the group identified as primary refractory DLBCL (19-21). For those patients displaying signs of partial if not complete response, following treatment intensification, a subsequent consolidation with auto-HSCT is a feasible and effective strategy, with variable survival expectancies, in a median of 40%, approximately. Thus, auto-HSCT should not be abandoned in this group of high-risk patients with early chemo-immunotherapy failure (19-20). Unfortunately, patients with fully refractory disease, progressing under induction chemo-immunotherapy and unable to obtain any reduction of tumor burden, have very few if any chance of response following auto-HSCT (21). In any case, prospective studies are required aimed towards increasing the response rate and ultimately to improve the adverse prognosis of patients with early chemo-immunotherapy failure. Recent results support the use of intensified therapy and auto-HSCT as the treatment of choice in primary refractory DLBCL patients. There are two phases of heightened vulnerability and therapy failure, where investigation of novel strategies is warranted: the pre-transplant management of a refractory disease and the risk of disease recurrence in the 6-12 months following autograft. Thus, auto-HSCT programs might represent the basis of prospective strategies combining novel drugs with intensified chemo-immunotherapy, in order to: i. provide high salvage therapy response rates and increase patient pool eligible for auto-HSCT; ii. offer additional post-autograft therapy aimed to prevent disease relapse following auto-HSCT.

II. relapsed DLBCL: The standard management of DLBCL patients who fail first-line therapy includes salvage chemotherapy followed by high-dose therapy and auto-HSCT. The PARMA trial demonstrated a significant survival advantage for relapsed patients with chemosensitive disease who were randomized to the high-dose therapy and autograft arm (22). Auto-HSCT is effective also for patients with relapsed DLBCL in the rituximab era and it remains the standard of care at least in patients with chemosensitive disease, recurring after at least 6-12 months since CR achievement. A recent survey from the EBMT has shown that for patients undergoing auto-HSCT more than 18 months after diagnosis, the 4-year Progression-free (PFS) and Overall Survival (OS) were 49% and 61%, respectively (23). The favorable impact of time from CR to disease relapse on the overall outcome following auto-HSCT has been further documented by an observational study conducted at Iowa University and at Mayo Clinic, USA (24). This recent report showed that DLBCL patients relapsing after 12 months from initial diagnosis had a 4-yr OS of 47% while those with transient or no response to initial therapy had a 4-yr OS of only 13%. Taken together, all these studies demonstrate that outcomes of relapsed or refractory DLBCL differ substantially when categorized by response to initial therapy and timing of relapse. The design and interpretation of uncontrolled trials should account for this heterogeneity in patients with relapsed DLBCL. Moreover, molecular studies may clarify whether DLBCL with late relapse had peculiar genetic features compared to fully refractory or early progressing DLBCL.

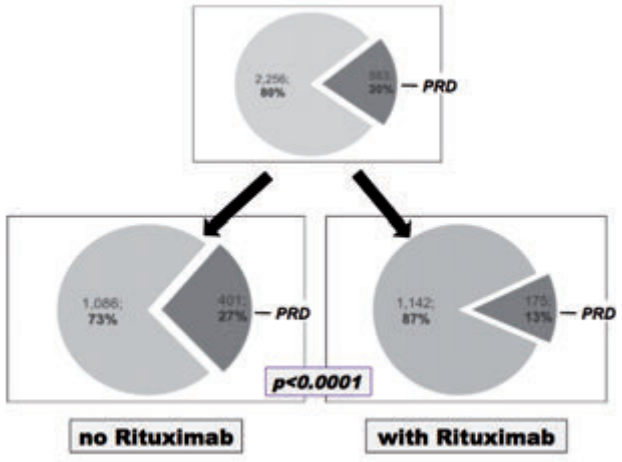

Figure 1. Incidence of Primary Refractory Disease in a large series of 2,839 patients with B-cell Lymphoma according to Rituximab administration.

Primary Refractory Disease (PRD) was defined as i. stable or progressive disease, following front- line therapy; ii. transient response with disease progression within six months, following first-line chemotherapy. Overall, 1,487 patients were treated in the pre-Rituximab era (no Rituximab), while 1,317 patients received Rituximab-containing chemotherapy (with Rituximab).

III. relapsed Follicular Lymphoma: Follicular Lymphoma (FL) is an indolent disease with a long-life expectancy. While disease recurrence is a frequent feature, primary refractoriness is unusual. Indeed, FL is characterized by a heterogeneous clinical outcome, most patients respond to induction chemo-immunotherapy with long survival, while less than 20 percent have reduced life expectancy due to early disease recurrence. The National LymphoCare study in USA documented a shorter OS for patients with early relapse compared to patient with chemo-sensitive disease, in accordance with previous observations by our group (16, 25). In these studies, patients presenting with late relapse, occurring at >2 yrs since diagnosis, have a long life expectancy and the use of treatment other than auto-HSCT should be preferentially considered. Within this context, several new, non-chemotherapeutic drugs are demonstrated to be safe and effective in FL (26). These drugs are particularly suitable for the management of FL patients in late relapse. On the other hand, patients with early relapse, known to be associated with short overall survival, high dose chemotherapy and auto-HSCT are believed to improve the outcome compared to conventional chemo-immunotherapy salvage programs (27). In fact, for early relapsing FL, the role of auto-HSCT should be further explored in comparison to the allogeneic transplantation approach (28).

IV. refractory/relapsed Hodgkin's Lymphoma: The treatment of Hodgkin lymphoma (HL) has improved with combined modality therapy, with approximately 80% of patients achieving long-term cure, with current poly-chemotherapies and additional radiotherapy if indicated. Unfor-

tunately, a fraction of approximately 20% of patients still suffers from refractory or relapsed disease. These patients require salvage chemotherapy. Several studies, both registry-based or multicentric randomized, have shown the high-efficacy of intensified therapy with auto-HSCT, with superior outcomes compared to salvage conventional chemotherapy (29-31). Based on these observations from studies of the last two decades, auto-HSCT has been extensively used as salvage therapy in HL. At present, auto-HSCT remains the treatment of choice in patients with relapsed or refractory HL after first-line therapy, and approximately 50% of patients can be cured [32]. Major advances have been achieved in the management of HL in the last few years, including the use of PET scan to strictly monitoring treatment response and the development of novel effective drugs, such as the anti-CD30 antibody-drug conjugate (ADC) brentuximab vedotin (BV) and anti-PD-1/PD-L1-L2 checkpoint inhibitors (1, 32-34). All these achievements will further improve the overall prognosis for HL patients and the role of auto-HSCT is expected to be gradually limited over time if not eventually abolished. However, additional studies and prolonged follow-up are needed to clarify the appropriate length of treatment, the durability of response and the ideal role of targeted drugs in the treatment strategy for HL. In addition, similar to Rituximab, these new drugs can be effectively combined with auto-HSCT programs. In particular, BV has been successfully employed either in the pre-transplantation phase to increase the proportion of patients eligible to autograft or following transplantation to reduce recurrence risks, as reported in recent trials (35-36). Thus, at present, auto-HSCT remains the standard of treatment for HL patients at the first treatment failure, with the possible addition of post-transplant BV consolidation in patients with particular high-risk features.

Conclusions: This brief review has discussed the role of auto-HSCT in different lymphoma subtypes where novel targeted drugs have obtained or are expected to obtain major clinical advances. Despite the introduction of these new drugs, auto-HSCT still have a distinct role, particularly in the salvage setting. The combination of auto-HSCT programs with novel drugs may foster and optimize the use of auto-HSCT in the management of high-risk lymphoma patients.

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